THE EFFECT OF THE MENSTRUAL CYCLE ON INVOLUNTARY DEHYDRATION FOLLOWING ENDURANCE EXERCISE

By

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KEY TO ABBREVIATIONS

ALDO	ALDOSTERONE
ANGII	ANGIOTENSIN II
CSK	COVERED SKIN TEMPERATURE
EL	EARLY LUTEAL PHASE
F	FOLLICULAR PHASE
LDBF	LASER DOPPLER BLOOD FLOW
LL	LATE LUTEAL PHASE
M	MENSTRUAL PHASE
NOC	NON-ORAL CONTRACEPTIVE
OC	ORAL CONTRACEPTIVE
PRA	PLASMA RENIN ACTIVITY
PRERD	PRE-RIDE
PROG	PROGESTERONE
POSTRD	POST-RIDE
PV	PLASMA VOLUME
RD	RIDE SECTION
RHYD	REHYDRATION SECTION
RPE	RATINGS OF PERCEIVED EXERTION
RST	REST SECTION
SBF	SKIN BLOOD FLOW
USK	UNCOVERED SKIN TEMPERATURE

VO_2 ....OXYGEN CONSUMPTION

Abstract of Dissertation Presented to the Graduate School
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THE EFFECT OF THE MENSTRUAL CYCLE ON INVOLUNTARY DEHYDRATION FOLLOWING ENDURANCE EXERCISE

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The overall objective was to determine whether physical performance and ability to voluntarily rehydrate are affected by phases of the menstrual cycle or oral contraceptives. Based upon the various changes in the hormones and enzymes responsible for hydromineral balance that occur across the menstrual cycle, there may be a phase of the cycle during which the subjects are able to more fully rehydrate themselves. In addition, women taking oral contraceptives may differ from normally cycling women in ability to rehydrate. In this study, 7 female recreational athletes (4 normally menstruating women and 3 women taking oral contraceptives) rode a cycle ergometer at 60-65% VO_2 max for 90 minutes or until exhaustion. Each subject performed 8 trials across 2 complete menstrual cycles. The trials occurred during four different points of the menstrual cycle; menstrual, mid-follicular, mid-luteal, and late-luteal phases. No fluids were given during the ride, but a 2-hour water rehydration period followed each trial. Performance was based on perceived difficulty of the exercise, body temperatures during exercise, weight loss during exercise, and measurements of plasma renin activity (PRA), angiotensin II (ANGII), and aldosterone (ALDO) concentrations. Sodium solution palatability and severity of thirst was assessed at several time points. The extent of involuntary dehydration was assessed by voluntary water intake during the rehydration period, body weight and change in plasma volume.

No changes in exercise performance across menstrual cycle phase were found in either NOC or OC subjects. The NOC group had increased PRA, ANGII, and ALDO levels during exercise in the luteal phase. The sodium palatability of one concentration was elevated during the menstrual phase. This result may be related to lower oxytocin levels at this time. This study also compared the exercise and ingestive responses of NOC and OC women. There were interesting differences indicated by the ingestive measures that suggest the OC group had slightly impaired rehydration and decreased sodium palatability. If oral contraceptive use reduces water intake or the acceptability of rehydration solutions containing sodium, it could delay rehydration or result in a cumulative fluid deficit with repeated exercise bouts. This could, in turn, impair performance.

INTRODUCTION

Overview of Study

Large amounts of data have been published concerning the responses to exercise of various systems involved in hydromineral regulation in humans. Only a minority of those studies has used female subjects, and even fewer have examined the effects of the phase of the menstrual cycle on these responses. Oral contraceptives also cause changes in these substances and in fluid balance, yet almost no research has looked at changes in water intake for humans. Consequently, nothing is known concerning a type of water deficit in women known as involuntary dehydration. Involuntary dehydration occurs when a person drinks water to satiety but still has a physiological water deficit. This research has important implications for the ability to thermoregulate appropriately during physical activity and/or heat exposure. Failure to fully recover from one episode of dehydration will impair heat loss and performance in subsequent exercise or heat exposures.

The following chapter gives an introduction to the physiological effects of exercise, especially as it relates to circulatory changes, thermoregulation, the endocrine system and effects of dehydration. It will also cover the mechanisms that control thirst, sodium appetite, and involuntary dehydration. Because this study focused on the influence of the menstrual cycle and oral contraceptives on endurance exercise and fluid intake, the reproductive hormones, the effect of endurance exercise on women, and their relationship to hydromineral balance will also be discussed.

Literature Review

Exercise

Exercise, whether recreational or as part of physical labor, poses several challenges to the maintenance of homeostasis in the body. Oxygen must be delivered to working muscles while carbon dioxide and other waste products are removed (Senay & Pivarnik, 1985). The endocrine system is activated to supply fuel to the working muscles and to participate in the cardiovascular adjustments. Fluid loss through sweat, evaporative heat loss, and movement of fluid out of the vasculature will result in a decrease of blood and plasma volume requiring cardiovascular adjustments in order to maintain blood pressure and cardiac output (Johnson, 1986; Morimoto, 1990). At the same time, heat is being produced by the contraction and relaxation of the active skeletal muscles and must be transported to the body surface and dissipated (Johnson, 1992). Circulatory and hormonal adjustments in vascular tension and blood flow attempt to balance these competing reflexes (Kenney & Johnson, 1992).

Brief/intermediate length exercise

With the onset of dynamic exercise, stimulation of sympathetic activity increases the rate and contractility of the heart, and the produces changes in circulatory resistance (Guyton, 1991). These circulatory adjustments, along with the increased venous return due to the pumping action of the active muscle, help to maintain arterial blood pressure, increase stroke volume, and results in an increase in cardiac output (Guyton, 1991).

Cardiac output is the amount of blood in liters pumped by the heart per minute, and is the product of heart rate and stroke volume. The increase in cardiac output is divided among competing nonthermoregulatory and thermoregulatory needs, and is balanced to best meet the needs of active skeletal muscle, maintain blood pressure, and increase cutaneous blood flow to maximize heat loss (Johnson, 1986; Johnson, 1992; Kellogg et al., 1991a).

The cardiovascular adjustments occurring immediately after the onset of dynamic exercise result in an overall shift in blood centrally. The distribution of blood flow during exercise is modified by both thermoregulatory and nonthermoregulatory reflexes (Kellogg et al., 1991a, 1991b; Kenney & Johnson, 1992). The thermoregulatory reflexes are responsible for ridding the body of the excess heat produced during exercise. Nonthermoregulatory reflexes optimize vascular volume, and serve in part to redirect blood flow from inactive to active areas so that cardiovascular functioning and working muscle perfusion can be maintained (Kellogg et al., 1991a, 1991b; Kenney & Johnson, 1992). The nonthermoregulatory reflexes dominate until the core body temperature increases above a temperature threshold, after which the thermoregulatory-driven adjustments are triggered (Johnson, 1986; Kellogg et al., 1991b).

One of the nonthermoregulatory reflexes engaged by exercise is the vasoconstriction evident in the cutaneous circulation during the first few minutes of exercise (Johnson, 1992). Nonacral human skin is innervated by both sympathetic active vasoconstrictor and active vasodilator nerves (Fox & Edholm, 1963; Kellogg et al., 1991a, 1991b). With the onset of muscular work, there is an almost instantaneous decrease in skin blood flow (Christensen et al., 1942; Kenney & Johnson, 1992). Studies in which one of the sympathetic components was selectively blocked showed that the initial decrease in cutaneous blood flow seen with exercise onset is due solely to the sympathetic active vasoconstrictor system (Kellogg et al., 1991b). By increasing cutaneous vasoconstriction and moving blood centrally, flow to active muscles, blood pressure, and cardiac output can be maintained (Christensen et al., 1942).

Supplying blood to active muscle is the other nonthermoregulatory reflex. As skeletal muscles begin work, heat, carbon dioxide, and waste products begin to build up in the tissue, while oxygen levels decrease. The vasculature in the working muscles dilates via active hyperemia, thus increasing local blood flow and allowing for greater product exchange (Guyton, 1991). Not only does blood flow increase in active tissue, but the total number of open capillaries increases and provides a greater surface area for product exchange (Senay & Pivarnik, 1985).

Working muscles produce a great deal of heat due to metabolic processes and friction of contracting muscle fibers. In humans, heat is transferred from the core to the skin by peripheral vasodilation and dissipates primarily thorough evaporation of sweat (Fortney et al., 1981; Fortney et al., 1984). When exercising in cool environments, several kilocalories of heat per liter of skin blood flow can be transferred from the core (Brengelmann, 1983). However, because of the initial cutaneous vasoconstriction, as exercise continues beyond 5 minutes or so there is a build-up of heat which results in an increase in core body temperature (Johnson, 1992). Feedback from central and peripheral thermoreceptors is integrated by the hypothalamus, primarily in the anterior preoptic region (Gordon & Heath, 1986; Johnson, 1992; Kenney & Johnson, 1992). After the core temperature is elevated beyond a certain threshold, a thermoregulatory stimulus for activating heat loss mechanisms is engaged (Johnson, 1986; Johnson et al., 1984; Kellogg et al., 1991b; Kenney & Johnson, 1992; Wyss et al., 1974).

In humans, heat loss mechanisms include the cutaneous vasodilator and sudomotor (sweat) systems. Skin blood flow increases rapidly during the first few minutes after vasodilation is initiated, then continues at a slower rate (Johnson 1992). This allows for heat to be transferred from the core to the skin surface and dissipated primarily by evaporation. However, the core temperature threshold during exercise is greater than the threshold under resting conditions (Johnson, 1986; Johnson, 1992; Johnson & Park, 1981; Kenney & Johnson, 1992). This shift to the right in the temperature threshold for increasing skin perfusion is due to a delay in the initiation of active vasodilation (Kellogg et al., 1991b). In the past it was hypothesized that this delay resulted from an increase in a proposed hypothalamic set point, controlling both vasomotor and sudomotor responses. However, behavioral research using subjects adjusting a water-perfused glove to their most preferred temperature found that the temperature was most closely related to their internal temperature and not affected by exercise (Cabanac et al., 1970). This indicated that the thermoregulatory set point was not changed during exercise. Subsequent physiological research has shown that while there is a delay in active vasodilation during exercise, there is not a delay in sudomotor activity onset (Johnson, 1992; Johnson & Park, 1981; Kellogg et al., 1991b). This suggests that there is a single set point for vasomotor and sudomotor control higher up, and that control diverges at some point allowing for modification of the vasodilator threshold without altering the sweating threshold (Johnson, 1992).

As previously mentioned, the total number of capillaries open increases during exercise. One consequence of increased capillary perfusion is that the surface area for fluid movement also increases (Senay & Pivarnik, 1985). With the fluid pressure and osmotic pressure changes that occur during cycle exercise, the Starling equilibrium for capillary exchange is upset and fluid is lost out of the intravascular space and moves into the extravascular space (Fortney et al., 1988). This results in a decrease of plasma volume by 7 to 15% during the first 10 to 20 minutes of exercise (Ekelund; 1967; Novosadova, 1977; Senay, 1979; van Beaumont et al., 1981). When short-term exercise is discontinued, plasma volume tends to return to pre-exercise values (Senay, 1979). If exercise is prolonged and significant fluid losses occur due to sweating, modifications of thermoregulatory skin perfusion and sweat rate takes place. Other nonthermoregulatory factors can also alter the threshold for vasodilation, the slope of skin blood flow per unit of core temperature, and the maximum cutaneous vasodilation in an effort to preserve cardiovascular functioning in the presence of homeostatic challenges. These factors include mode of exercise, level of hydration, skin temperature, and plasma osmolality (Brengelmann, 1983; Nadel et al., 1980; Johnson, 1986; Johnson, 1992; Johnson et al., 1984; Johnson & Park, 1981; Kenney & Johnson, 1992; Montain & Coyle, 1992a, 1992b; Morimoto, 1990).

The endocrine system has an important role in the body's adaptation to onset of exercise and in the ability to sustain exercise. With exercise onset, activation of motor centers in the brain and impulses from the working muscles stimulate the sympathetic system, adrenal activity, and pituitary hormone release (Galbo, 1985). The release of hormones such as epinephrine and norepinephrine participate in the cardiovascular changes (e.g. vasoconstriction and bronchodilation). During moderate to heavy exercise growth hormone, cortisol, epinephrine and norepinephrine all increase, while insulin decreases (Galbo, 1985). These work together to maintain plasma glucose concentrations while still providing fuel for the working tissues (Powers & Howley, 1994).

Another subset of the endocrine system is concerned with adjusting to the fluid and electrolyte changes and losses that occur during exercise. In general, the endocrine response to hydromineral changes and the magnitude of this response depends on the relative intensity of the exercise more than the training level of the individual (Convertino et al., 1983; Galbo, 1985). With exercise onset, the redistribution of blood flow results in decreased renal perfusion. At moderate-intensity exercise the increase in blood pressure and capillary perfusion force fluid out of the intravascular space decreasing blood and plasma volume and increasing plasma osmolality. These changes shift the body into a mode of water and sodium conservation (Convertino et al., 1981; Fellmann, 1992). Renin, angiotensin II (ANGII), aldosterone (ALDO), and antidiuretic hormone (ADH) are substances which not only help in hydromineral conservation, but also are important in repairing fluid losses by initiating water and sodium intake. Renin is an enzyme, formally associated with the kidney, but now known to be present in many other tissues. This enzyme cleaves angiotensin I into ANGII. Angiotensin II is a vasoconstrictive hormone that also has potent dipsogenic actions. Increases in ANGII plasma levels stimulate the release of ALDO from the adrenal cortex. Aldosterone is a mineralocorticoid important in conserving sodium. The posterior pituitary hormone ADH prevents water loss from the kidney. One study had indicated that ADH can decrease sweat loss, either by decreasing skin blood flow or direct action on the sweat gland (Nicholson et al., 1995). The effects of these hormones on hydromineral balance will be discussed in the Thirst section.

Endurance exercise

When an exercise bout continues beyond 60 minutes without fluid replacement, fluid lost via sweat begins to challenge the cardiovascular system, which in turn affects thermoregulation. Whereas dehydration from fluid deprivation results in both intracellular and extracellular fluid losses, fluid losses from sweat initially result in extracellular fluid losses (Johnson & Thunhorst, 1997). Sweat is a hypotonic, protein-free fluid drawn out of the intravascular space. However, the hypotonic fluid losses increase the osmolality of the extracellular fluid and result in water losses from the intracellular compartment as fluid shifts to compensate. The electrolyte concentration of the fluid depends on sweat rate and the acclimatization of the person, and can range from 5 mEq to higher than 60 mEq per liter (Guyton, 1991). At slower sweat rates or in an acclimatized person, most of the sodium and chloride ions will be reabsorbed as the fluid flows through the sweat gland. At high sweat rates or in an unacclimatized person, the total ion concentration will be much greater. Increased ALDO secretion is responsible for the greater sodium conservation following acclimatization. Sweat losses increase the osmolality and decrease the plasma volume, both of which result in cardiovascular and thermoregulatory changes. Dehydration and decreases in blood volume are associated with an elevated core temperature during exercise (Armstrong et al., 1997; Greenleaf & Castle, 1971; Nadel et al., 1980; Nielsen, 1974; Sawka et al., 1985). This elevation in core temperature results from a decrease in the activity of heat dissipating mechanisms, especially cutaneous perfusion and sweating, which occurs with dehydration. If exercise continues and more fluid is lost from the vascular space, stroke volume will begin to fall (Brandenberger et al. 1986; Brengelmann, 1983; Nose et al., 1988). Stroke volume is the amount of blood pumped by each ventricular contraction. In an attempt to maintain cardiac output, the heart rate of the subject increases (Ekblom, 1970; Ekelund, 1967). This increase in heart rate and stroke volume, which is usually accompanied by a decrease in circulatory pressure, has been termed "cardiovascular drift" (Rowell, 1974).

Decreased vascular volume may modify skin blood flow through baroreflex control. Low blood pressure or decreased cardiac filling is sensed by arterial and cardiopulmonary baroreceptors. Baroreceptors send signals centrally through the vagus and glossopharyngeal cranial nerves to the nucleus tractus solitarius (NTS) in the medulla (Guyton, 1991). When blood pressure decreases the baroreceptors are relaxed and their rate of firing is reduced. Efferent signals from the NTS then stimulate sympathetic activity, which increases peripheral vascular resistance and heart rate to maintain blood pressure. Noradrenergic projections from the NTS act synergistically with projections sensitive to hormonal information, stimulating forebrain structures important in compensating and recovering from an extracellular fluid deficit (Johnson & Cunningham, 1987). Baroreceptor unloading also stimulates increased release of substances with vasoconstrictive actions such as renin and ADH. These substances also promote water and sodium retention and help to expand plasma volume (Smith & Kampine, 1990)

Several studies have obtained results compatible with the idea of baroreflex control of skin blood flow during exercise. Nadel et al. (1980) found that the threshold for cutaneous vasodilation was elevated and maximal skin blood flow was significantly reduced during exercise in hypohydrated conditions. Cardiac output was lowest in the hypohydrated group when compared with euhydrated or hyperhydrated subjects, and continued to fall throughout exercise. It is probable that baroreceptors initiate a response designed to maintain cardiac filling pressure by shifting blood centrally from the peripheral vasculature. Fortney et al. (1981) found that subjects made isotonically hypovolemic prior to exercise exhibited a decrease in sweat rate at a given core temperature. The hypovolemic subjects also had less vascular fluid lost during exercise. These results suggest that mechanisms are engaged to defend central blood pressure. A study in which lower body negative pressure (LBNP) was applied to exercising subjects found that the rise of and the peak forearm vascular conductance was attenuated compared to exercise without LBNP. This attenuation was reversed quickly when baroreceptor unloading was stopped (Mack et al., 1995). Exercise in the water, which provides pressure to shift blood volume centrally, is characterized by a continuous rise in skin blood flow instead of the plateau normally seen (Nielsen et al., 1984). Kellogg et al. (1990) selectively blocked vasoconstrictor sympathetic response during LBNP and found that cutaneous vascular conductance was significantly decreased. This indicates that baroreceptor unloading reduces cutaneous perfusion through control of the active vasodilator system. These and other studies provide evidence that baroreceptors, and hence, blood volume and blood pressure play a role in the modification of skin blood flow during prolonged exercise. The decrease in skin blood flow during exercise results in an impairment of the heat dissipation mechanisms and increases in core temperature.

In addition to hypovolemia, hyperosmolality also effects the circulatory and thermoregulatory responses during exercise. Fluid loss from sweat can be as great as several liters an hour in an acclimatized person (Brengelmann, 1983; Guyton, 1991). As previously mentioned, the sodium content of sweat will vary with acclimatization. Isotonic fluid is taken primarily from the extracellular fluid, while pure water is removed from both the extracellular and intracellular fluid (Brengelmann, 1983). Increased plasma osmolality from sweat losses or movement of intravascular fluid into the interstitial space, is sensed by osmoreceptors located both centrally and peripherally (Bouque et al., 1994). An in-depth explanation of how these receptors function will follow in the Thirst section. When osmolality is increased, efferents are activated, and neural and hormonal mechanisms designed to conserve fluid respond. Fortney et al. (1984) found that subjects with normovolemic hyperosmolality had elevated thresholds for cutaneous vasodilation and sweating during exercise. Hyperosmolality may mediate its effects on thermoregulatory mechanisms by direct action in the hypothalamus or through the activation of hormones such as ADH. When hypovolemia is combined with hyperosmolality the osmotic threshold for ADH release is lowered.

Mechanisms of Thirst and Sodium Intake

Water intake to replace the fluid losses from sweat will help to maintain cardiovascular and thermoregulatory functions, and extend the duration of exercise. Water and sodium are critical to the life and normal function of the body, and yet both are continually lost even in the non-exercising organism (Cannon, 1918). Without water an animal will die within a few days. Because of this the body is equipped with a series of neural and humoral systems which sense osmolality or volume changes in body fluids, and initiate circulatory and behavioral responses to bring the body back into homeostasis.

Thirst, sodium appetite, diuresis, and natriuresis are the primary controllers of hydromineral balance (Johnson & Thunhorst, 1997).

Water in the body is divided into the intracellular and extracellular fluid compartments. The extracellular compartment is in turn made up of intravascular and interstitial water. If the fluid content of either compartment deviates outside of the normal range, mechanisms are activated to correct the error (Fitzsimons, 1961a, 1961b). Research earlier in this century focused primarily on increased osmotic pressure and cellular dehydration as driving thirst. Gilman (1937) showed by injecting hypertonic concentrations of impermeable solutes that it was the water content of the cells that was important, not just an increase in osmolality. Several years later the term osmoreceptors was proposed as a term describing the specialized cells that act as intracellular deficit sensors (Verney, 1947).

Intracellular thirst

Osmoreceptors are located both peripherally and centrally. Splanchic osmoreceptors are probably located in or near the hepatic portal vein. Studies suggest that this location allows the osmoreceptors to provide a rapid "front line" defense against the osmotic change which result from absorption of ingested solutes or water (Chwalbinska-Moneta, 1979). A quick adjustment to hepatic osmotic changes permits the maintenance of a relatively stable systemic osmolality, rather than an oscillation of hyper- or hypotonicity every time the animal eats or drinks. Spalanchic osmoreceptors send signals through the hepatic vagal afferents, probably projecting to the NTS and ventrolateral medulla before traveling to the anteroventral third ventricle (AV3V) region.

Central osmoreceptors are more important in monitoring the systemic osmolality of the body. Current research places central osmoreceptors in the AV3V region of the brain, most notably in the organum vasculosum of the lamina terminalis (OVLT).

The OVLT and other structures, such as the subfornical organ (SFO) and the median preoptic nucleus (MePO), also are known as circumventricular organs (CVO). The circumventricular region is devoid of a blood-brain barrier, which allows for relatively free sampling of the peripheral blood composition. The structures comprising the CVO are widely interconnected with one another. In addition, projections from the SFO and MePO nucleus innervate the supraoptic nucleus (SON) and paraventricular nucleus (PVN). The magnocellular neurons located in the latter two nuclei manufacture ADH and oxytocin (OXY), and when stimulated release these hormones from the posterior pituitary. In the past decade, researchers have begun mapping immediate early genes as a marker of neuronal activity, allowing them to determine areas of activity during various physiological conditions. This method has recently produced evidence suggesting that the PVN and SON also are osmoreceptive and can be stimulated by hyperosmolality independently of the SFO or OVLT (Han & Rowland, 1996).

Osmoreceptors send afferent signals in response to changes in their size. Under euhydrated conditions, osmoreceptors stimulate a low baseline amount of activity. This tonic ADH secretion is sufficient to concentrate urine to half of the maximal level (Robertson, 1987). If the plasma is hypotonic, the osmoreceptor cell size increases and afferent signals are suppressed. This results in inhibition of ADH to below detectable levels and diuresis. If the plasma is hypertonic and the cell size decreases, then afferent signals increase and stimulate ADH release. High ADH levels restrict body water loss by producing a concentrated urine, and in humans, decreasing sweat losses. Activation of the AV3V region generates the appropriate physiological and behavioral responses (drinking) to repair water deficits during hypertonic conditions.

Extracellular thirst

While osmoreceptors monitor and initiate the appropriate responses to intracellular fluid challenges, another physiological system exists to monitor and maintain the extracellular fluid volume. Following the early studies on osmoreceptors and intracellular dehydration, researchers suggested that the expansion of osmoreceptors, as seen with sodium depletion, also stimulated thirst. However, Stricker (1966) showed that a decrease in extracellular volume, with no change in osmoreceptor size, was able to elicit water intake. The extracellular thirst system uses volume and pressure receptors in the vasculature and kidney to monitor the status of the extracellular fluid compartment. The baroreceptors and volume receptors signal cardiovascular, humoral, and neural (physiological and behavioral) responses to restore extracellular fluid to normal range. The anatomy and function of these receptors was discussed in a previous section. “Unloading” the receptors, by constricting the inferior vena cava or obstructing blood flow by an inflated balloon, produces increases in water intake and decreases urine and electrolyte excretion, and increases plasma renin activity (PRA) and ADH levels (Moore-Gillon & Fitzsimons, 1982). Fitzsimons and Moore-Gillon (1980) found that blocking the vagal sympathetic nerve that carries the afferents from baroreceptors and volume receptors enhances these responses to hypovolemia. Under normal conditions, these receptors probably send inhibitory stimulation centrally, which is removed when the receptors are unloaded during hypovolemia. Essentially, when the intravascular volume is low, the receptor afferents signal central areas that, in turn, stimulate sympathetic activity to maintain blood pressure. The sympathetic activity also stimulates the renin-angiotensin-system (RAS) and the release of ADH and ALDO. The RAS is a strong stimulator of ADH release. Baroreceptors, probably arterial, are also capable of directly increasing ADH release (Chen et al., 1993; Thrasher et al., 1982; Thrasher, 1994). Strong hemorrhagic shock causes large plasma increases in the hormone, even with the RAS blocked (Morton et al. 1977). The RAS and ADH response is critical for conserving water and sodium to protect the extracellular fluid. The vasoconstrictive effects of both hormones also helps to raise blood pressure back toward normal. When the baroreceptors and volume receptors are stretched and their afferent firing increases, the opposite responses occur--diuresis and natriuresis (Fater et al., 1982; Moore-Gillon & Fitzsimons, 1982). The kidney also has mechanisms to monitor and respond to changes in extracellular fluid volume. Decreased pressure in the afferent arterioles, increased sympathetic activity, and low NaCl concentration in the macula densa of the nephron, all stimulate renin secretion, and consequently, activate the peripheral RAS.

Not only does the peripheral RAS work to conserve the remaining fluid, but it is an important signal in initiating thirst and sodium appetite and returning the extracellular fluid volume to a normal level. In a series of studies conducted in the 1960s, Fitzsimons found that reducing renal blood flow by ligation of the vena cava, constriction of the renal arteries or the aorta above the kidney, caused drinking in rats (Fitzsimons, 1964, 1967). He hypothesized that kidney renin was responsible for the increase in drinking because nephrectomy prevented drinking in hypovolemic rats, but injecting normal rats with renal cortex extracts caused them to drink (Fitzsimons, 1966, 1967, 1969). The injection of ANGII intracerebrally also was found to produce drinking, and the discovery of renin in the brain tissue of nephrectomized dogs, led to the identification of a cerebral RAS (Fitzsimons & Rolls, 1970; Ganten et al., 1971).

Central mechanisms.

The brain areas important in ANGII-stimulated drinking are the areas previously discussed in relation to cellular thirst: the CVOs. Injection of low doses of ANGII into the SFO or AV3V produces drinking, while lesions in these areas reduce water intake (Buggy & Johnson, 1978; Simpson & Routtenberg, 1973). However, as previously mentioned these structures are widely interconnected, so that the lesions destroy not only the cell bodies but also any fibers of passage. Research using immediate early gene mapping as an indication of neuronal activation, has found that the SFO seems to be the primary site for sensing and initiating a response to peripheral ANGII (Rowland et al., 1994b, 1994c). Tracing studies have also shown a large descending angiotensinergic projection from the SFO. Immediate early gene expression studies indicate these project to the OVLT and MePO in the AV3V region, and the SON and PVN (Rowland et al., 1996). Noradrenergic afferents stimulated by brain regions receiving blood pressure and volume information, such as the NTS, project to the MePO area (Johnson & Thunhorst, 1997). FOS studies suggest that pressure/volume-related information may be sent to the PVN and SON also (Rowland, 1994a). It appears that humoral information from the peripheral RAS is sensed by the SFO and integrated with cardiovascular information from the baroreceptors and volume receptors (Thunhorst & Johnson, 1993; Wilson et al., 1984). This integration probably occurs in the MePO area, with noradrenaline and ANGII each facilitating the others effect on thirst motivation (Johnson et al., 1996; Zardetto-Smith et al., 1993).

Sodium intake

Many of the hormones and brain structures discussed in relation to thirst are also important in sodium intake. Current research indicates that sodium intake is governed by the addition or subtraction or both excitatory (RAS and ALDO) and inhibitory (baroreceptor, OXY, and atrial natriuretic peptide) signals. For many years it was thought that the peripheral RAS did not play an important role in inducing sodium appetite other than stimulation of ALDO secretion. Epstein proposed that it was the ALDO acting synergistically with central angiotensin which produced salt appetite (Sakai et al., 1990; Yang & Epstein, 1991). It has since been shown that converting enzyme inhibitor (CEI) blockade of the peripheral RAS, while leaving the cerebral RAS unblocked, is sufficient to suppress intake in sodium depleted rats (Thunhorst & Fitts, 1994). This indicates that the peripheral system is vital to depletion-induced sodium appetite, and may activate the central RAS to stimulate sodium appetite. Sodium intake in response to ANGII administration is greater when rats are pre-treated with ALDO, lending support to the synergy hypothesis (Fluharty & Epstein, 1983). Again, lesion and CFOS studies have implicated the CVO as the site for transducing the peripheral ANGII signals in the brain (Vivas & Chiaraviglio, 1992; Vivas et al., 1995; Weisinger et al., 1990).

Baroreceptor input may also help regulate sodium intake (Toth et al., 1987). It is suggested that during hypervolemia, the increase in baroreceptor input to the AV3V region stimulates the cerebral atrial natriuretic peptide (ANP) system, which stimulates the release of OXY from the posterior pituitary (Antunes-Rodrigues et al., 1997). The central oxytocinergic system then stimulates the release of peripheral ANP from the atria of the heart. If the AV3V region or the posterior pituitary is lesioned, the ANP response to hypervolemia is blocked (Antunes-Rodrigues et al., 1991). Atrial natriuretic peptide inhibits the RAS and ALDO secretion, thus promoting sodium and water loss and reducing blood volume (Goodman, 1994). Oxytocin also has a strong natriuretic action, and together with ANP, is associated with inhibition of sodium intake (Blackburn et al., 1995; Sjöquist et al., 1995; Verbalis et al., 1991). Conditions that are associated with a decrease in sodium appetite (i.e. hyperosmolality) are associated with increases in the release of OXY from the posterior pituitary. Conditions that are associated with sodium appetite (e.g.. sodium deprivation), are associated with decreases in OXY release (Stricker & Verbalis, 1987). When made hypovolemic, rats first ingest water until they become hypotonic. Hypotonicity suppresses both ADH and OXY release. Some time after this, sodium appetite will develop (Stricker, 1981; Stricker & Verbalis, 1986). It is not yet known if baroreceptors participate in stimulating sodium intake during hypovolemia, but recent studies suggest that they may. Rats with lesions of the ventromedial nucleus of the hypothalamus (VMH) increased their saline ingestion after ANGII injection into the AV3V (Camargo et al., 1991). The VMH, a structure which has connections with the AV3V area, also is known to have pressor activity (Gauthier et al., 1981; Johnson et al., 1981; Valladao et al., 1992).

Integration of thirst and sodium intake systems

The intracellular and extracellular thirst systems work together to control water intake and body fluid balance. During dehydration, both systems are engaged by an increase in plasma osmolality accompanied by a fall in plasma volume. Under normal conditions, change in osmolality is a much more sensitive stimulus than volume change. An increase of only 2% in plasma osmolality will trigger almost maximal ADH release and water conservation, while a much greater decrease in blood volume (10%) must occur to stimulate ADH release (Robertson et al., 1976). However, moderate hypovolemia does sensitize osmolality as indicated by a lowered osmotic threshold for ADH release. Hypovolemia stimulates the peripheral RAS and ALDO release. This promotes sodium and water conservation, raises blood pressure in an attempt to compensate for the decreased blood volume, and by activating the central RAS can initiate water intake to repair deficits. Also, the RAS may be responsible for the lowered osmotic set point during moderate hypovolemia (Andersson et al., 1980). Both the oral stimulation of water intake and the post-absorptive decrease in osmolality quickly suppresses ADH secretion, even if the blood volume deficit remains (Geelen et al., 1984; Morian & Rowland, 1995; Seckl et al., 1986). If water for intake is not accessible, sodium excretion occurs to reduce the hyperosmolality (Weisinger et al., 1985). Therefore, under normal, "non-emergency", conditions plasma osmolality is maintained at the expense of extracellular fluid volume. If blood volume depletion continues or is severe enough the RAS is activated, and a vigorous water intake initiated until the osmotic stimulus for drinking is removed. After a longer period of time, sodium intake will be stimulated to help retain the extracellular fluid. Alternating water and sodium intake until the volume deficit is repaired and osmolality is back to normal will bring the body's hydromineral balance back into homeostasis.

Women and Exercise

The increasing presence of women in military operations, physically demanding jobs, and sports has necessitated an examination of the interaction between phases of the menstrual cycle and physical performance. The following section will review the physiology of the menstrual cycle and oral contraceptives. Although there is little research available on fluid intake in women, the effect of the ovarian hormones on hydromineral balance and exercise performance will be discussed.

Menstrual cycle

The menstrual cycle of women takes an average of approximately 28 days to complete, and is divided into several different functional phases. For convention, this paper and the following experiments will consider onset of the menstrual phase as Day 1 and the start of a new cycle. The menstrual phase lasts for 3 to 5 days, during which the lining of the uterus sloughs off. This is in effect the end of the previous cycle and the beginning of the next. With the onset of menstruation the levels of follicular stimulating hormone (FSH) increase, followed several days later by an increase in luteinizing hormone (LH) concentrations. Follicular stimulating hormone stimulates the growth of several follicles over the next week (follicular phase), with one secreting more estrogen than the others and becoming the mature follicle. The increase in estrogen secretion triggers a large release of both FSH and LH from the anterior pituitary. Luteinizing hormone causes the follicle cells to begin secreting more progesterone and less estrogen, and acts with FSH to cause the mature follicle to swell. About 16 hours following the LH peak ovulation occurs; the follicle ruptures and the ovum is released into one of the fallopian tubes. Ovulation typically occurs at approximately Day 14 and marks the halfway point of the menstrual cycle. The luteal phase now begins and lasts until menstruation initiates the next cycle.

Whereas the menstrual and follicular phases were dominated by the maturation of the follicle, the luteal phase is dominated by the development of the corpus luteum. The corpus luteum is a mass made up of the follicle's remaining cells. Due to the effect of LH on the corpus luteum, it begins to secrete large amounts of progesterone and estrogen. The levels of these hormones begin to increase slightly prior to ovulation, after which they increase greatly. Although estrogen is elevated during both the follicular and luteal phases, progesterone levels are nominal during the follicular phase, < 3 ng/ml. The high levels during the luteal phase, > 10 ng/ml, indicate the presence of a functioning corpus luteum and can, therefore, be used as an indication of ovulation (Guerrero et al., 1976; Yussman & Taymor, 1970). Peak blood concentrations for both hormones are reached at the mid-luteal phase, around Day 21.

During the follicular phase the high estrogen levels cause the uterine endometrium to thicken. In the luteal phase progesterone further prepares the lining for implantation and nutrition of a fertilized ovum. If fertilization has not occurred by approximately Day 26 of a woman's menstrual cycle, the corpus luteum begins to disintegrate and the progesterone and estrogen levels decrease. The removal of the hormonal stimulation, along with the release of vasoconstrictive substances, leads to the degeneration and sloughing off of the uterine lining which is then dispelled through menstrual bleeding. The high levels of progesterone and estrogen in the luteal phase have an inhibitory effect on the release of FSH and LH. When these levels fall to very low concentrations approximately 2 days before the onset of menstruation, the suppression on the anterior pituitary hormones is removed. Consequently, the levels of FSH and LH start to rise around the time of menstruation, and the next cycle begins.

Oral contraceptives

Since their introduction in the 1960s, oral contraceptives have become one of the most widely taken prescription medications. Not only does their use prevent pregnancy, but may also protect against anemia, bone density loss, and is associated with a decreased incidence of uterine and ovarian cancer. The majority of oral contraceptives used today are known as combination pills because they contain both an estrogenic and progestational component. Synthetic derivatives are used due to the rapid inactivation of the natural hormones when taken orally. Administration of these hormones suppresses both FSH and LH release, thus preventing ovulation and creating an unfavorable uterine environment for fertilization and implantation. Preparations differ in the type of synthetic hormones used and in their dosage. While ethinyl estradiol is usually the type of estrogen used in combination oral contraceptives, there are a variety of progestins utilized. Oral contraceptives can be delivered in a monophasic, biphasic, or triphasic fashion. Monophasic oral contraceptives have the same ratio of hormones across the menstrual cycle. In biphasic and triphasic oral contraceptives the ratio of progestin to estrogen changes from a lower progestin dose early in the cycle, to higher progestin doses later in the cycle. The triphasic preparation most closely mimics the hormone profile across the normal menstrual cycle and reduces the total amount of progestin given (Thomas & Keenan, 1986).

Ovarian steroid effects on hydromineral balance

Estrogen and progesterone also have effects on water and electrolyte balance and influence the levels of the hormones important in regulating hydromineral balance. Because of its chemical similarity to ALDO, endogenous estrogen has a slight effect in the promotion of sodium and water retention (DeVries et al., 1972). Exogenously administered estrogens, such as ethinyl estradiol, exert an even stronger fluid retaining effect. Progesterone produces an antimineralocorticoid effect by competing with ALDO for receptors in the kidney tubules. Although progesterone has a slight ALDO-like effect, this effect is much weaker than that of ALDO and subsequently leads to a net loss of sodium and water. Plasma and serum sodium has been found to be significantly lower in the luteal phase, probably due to progesterone's natriuretic effect (Chapman et al., 1997; Olson et al., 1996). The older generation of synthetic progestins did not have the ALDO antagonistic activity of natural progesterone, and some actually potentiate estrogen's effects on fluid balance. However, recently developed progestins mimic natural progesterone's physiological actions much more closely and do stimulate the natriuresis seen during the normal cycle.

The reproductive hormones not only influence fluid and electrolyte balance by direct renal action, but also play a role in the cyclic regulation of other hormones important in hydromineral balance. As mentioned in the discussion of the menstrual cycle, estrogen dominates the follicular phase. Forsling et al. (1981) found that the plasma levels of ADH peaked around the time of ovulation, soon after the rise in estrogen. Antidiuretic hormone levels reached a nadir with the onset of menstruation when estrogen and progesterone are also low. Other studies have found a peri-ovulatory peak in ADH levels when endogenous estrogen levels are high in humans and rats, or when estrogen was administered to postmenopausal women (Forsling & Peysner, 1988; Forsling et al., 1982; Punnonen et al., 1983). Although the levels of OXY show much more variability across the menstrual cycle and between individuals, some researchers have been able to identify a similar pre-ovulatory peak in the plasma hormone level (Kumaresan et al., 1983; Windle & Forsling, 1993). Other studies have produced conflicting results, with an elevation in OXY during high estrogen in some women but not in others (Stock et al., 1991). The mechanisms controlling these hormonal variations are unclear. ADH and OXY may be responding to fluid and electrolyte changes that are associated with estrogen, such as an increase in sodium retention or a decrease in food and water intake (Forsling & Peysner, 1988). Alternatively, estrogen may directly affect their release or modify the central mechanisms of fluid balance (Findlay et al., 1979; Forsling et al., 1981).

As previously mentioned, the high progesterone levels during the luteal phase induce natriuresis. It is hypothesized that this sodium loss activates the RAS which is then followed by an increase in ALDO release (Gray et al., 1968; Sundsfjoid & Aakvaag, 1970). Supporting this idea is the finding that during an anovulatory cycle or if ovulation has been suppressed by oral contraceptive administration, no peak in PRA or ALDO is observed (Gray et al., 1968; Michelakis et al., 1975). Additionally, a recent study by

Braley et al. (1996) showed that progesterone did not directly stimulate ALDO secretion, but indirectly stimulated the RAS and ALDO secretion in a manner similar to sodium restriction.

Antidiuretic hormone levels fall throughout the luteal phase, reaching the lowest levels with the onset of menstruation. Forsling et al. (1982) found that administration of a synthetic progesterone did not affect ADH concentration; but when given in conjunction with estrogen, mimicking the hormonal state observed during the mid-luteal phase, a fall in ADH concentration occurred. However, the results of several studies have indicated that the osmoregulatory set point is lowered during the luteal phase. By injecting hypertonic saline into recumbent subjects during different cycle phases, Spruce et al. (1985) found that the osmotic threshold for ADH release and the plasma osmolality at which thirst is recognized are both lowered during the luteal phase. Resetting the osmoregulatory threshold downward and lowering the basal plasma osmolality could lead to a slight water retention during the luteal phase (Vokes et al., 1988).

A couple of studies, however, suggest that the opposite may be true, and that plasma volume may actually be contracted during the luteal phase. Turner and Fortney (1984) measured daily plasma volume change across the menstrual cycle and found it was lower during the luteal than in the follicular phase. Trigoso et al. (1996) measured baseline hormone levels and the response to hypertonic saline infusion in seated women during the follicular and luteal phases. Contrary to other studies, these researchers found no significant differences in serum sodium, plasma osmolality, ADH and thirst levels. They did find that ANP levels were higher and expressed a greater response to hypertonic saline in the follicular phase. These results, along with the activation of the RAS and increased ALDO found in the luteal phase, may indicate a decrease in plasma volume. Other researchers believe that because of increased capillary permeability in the later half of the menstrual cycle, fluid may shift out of the vascular space and into the interstitial space (Bisson et al., 1992; Shanholtz et al., 1997). Experimental procedures may help explain the difference in results regarding osmoregulation, ADH, and plasma volume. Various studies have used fed or fasted subjects, and supine versus seated positions for the blood draws. Plasma volume can decrease by 4.8% in the first five minutes after moving from a supine to a seated position (Pivarnik et al., 1986).

Oral contraceptive administration also alters hydromineral hormone levels. The stimulatory effect of oral contraceptives on the RAS is well documented. Synthetic estrogens are more potent than endogenous estrogen on increasing the synthesis of angiotensinogen, the prohormone of ANGII (Oelkers, 1996). Although the plasma levels of PRA and ANGII do increase slightly with oral contraceptive use, the elevation is normally limited by the feedback inhibition of increasing ANGII levels on renin secretion. The progestins without the natriuretic effect of natural progesterone cause no further activation of the RAS and ALDO. The newest progestins, such as Drospirenone, have antimineralocorticoid properties that lead to a significant increase in PRA, ANGII, and ALDO secondary to sodium losses (Oelkers et al., 1995). The estrogen component of oral contraceptives has a stimulatory effect on ADH concentration, but progesterone counteracts this effect in the combination pills (Bossmar et al., 1995). Oral contraceptive administration does suppress the ADH peak that is normally seen around the time of ovulation (Hauksson et al., 1987). Oxytocin and ANP concentrations have been found to increase with estradiol alone or combination oral contraceptive treatment (Bossmar et al., 1995; Oelkers et al., 1995; Silber et al., 1987; Uvnas-Moberg et al., 1989). With the use of a combined monophasic oral contraceptive, Tollan et al. (1992) found that plasma volume and capillary permeability was increased, and suggested this was due to the estrogen component.

In summary, estrogen and progesterone influence hydromineral balance across the menstrual cycle. The increase in ADH at ovulation and the increase in RAS activation and ALDO secretion in the luteal phase have been verified by several studies. Some researchers have also found changes in OXY, ANP, osmoregulation, and plasma volume, but the presence and direction of changes in these variables needs further study. In addition, oral contraceptives exert a similar effect on these hormones and fluid balance. However, the magnitude of these effects is, in general, greater than that of the natural ovarian steroids.

Ovarian steroids and fluid intake

Ovarian steroids have also been found to affect water and sodium intake in some animals. It has been noted in female sheep and rats that water and sodium intake is reduced during estrous and that ovariectomy abolished the cyclic intake (Danielsen & Buggy, 1980; Findlay et al., 1979; Michell, 1975). Intake elicited by the administration of substances associated with extracellular thirst regulation, such as ANGII, is also attenuated around the time of estrous in rats (Findlay et al., 1979). They suggested that ovarian hormones alter fluid intake through extracellular mechanisms, and indeed, did not find any alteration in cellular thirst. In a series of experiments, Fregly and others found the chronic administration of estrogen, alone or with progesterone, reduced the dipsogenic potency of peripheral and central administration of ANGII (Fregly, 1980; Fregly et al., 1985; Fregly & Thrasher, 1978). However, Thrasher and Fregly (1977, 1978) did note a decreased water intake to hypertonic saline infusion in estrogen-progesterone treated rats, but not to dehydration. Progesterone treatment alone did not affect water intake. Both endogenous and exogenous estrogen also seem to inhibit sodium appetite (Scheidler et al., 1994).

In general, the studies that have examined estrogen and fluid intake, have not examined spontaneous water intake during chronic hormone treatment. When they measured the daily intakes of rats treated for 14 and 28 weeks with estradiol benzoate, Carlberg et al. (1984) found that the rats had a significantly higher daily water intake, yet still showed an attenuated response to dipsogens. Because this effect may take several weeks to manifest, future studies attempting to uncover the mechanisms by which estrogen affects intake will need to resolve how to define "chronic" estrogen treatment. The cyclic rise and fall of estrogen both naturally and with oral contraceptive use may exert a physiological effect more similar to short term estrogen treatment.

The idea that estrogen acts centrally to influence thirst mechanisms has been supported by several studies. Jonklaas & Buggy (1985) found that perfusion of ANGII into the MePO area of estrogen treated rats produced a depression in water intake. In addition, ANGII binding was decreased in the MePO and in tissue containing the AV3V and SFO areas following estrogen treatment. Other studies have found that the number of ANGII receptors in the anterior pituitary fluctuates across the estrous cycle and is reduced with estrogen treatment (Seltzer et al., 1992). Experiments utilizing brain slice preparations from estrogen-treated rats have reported a decrease in firing of AV3V neurons to hypertonic and ANGII perfusion (Akaishi & Homma, 1996). New techniques, such as immediate early gene mapping, may help future research to define the role of estrogen in fluid intake.

Women and exercise performance

Most studies have found no significant effect of phase of menstrual cycle on the performance of endurance exercise. Increased heart rates, VO_2 per work load, and ratings of perceived exertion (RPE) have been reported by some researchers during luteal phase exercise (Hessemer & Brück, 1985; Higgs & Robertson, 1981; Pivarnik et al., 1992). However, others have found no difference in heart rate, VO_2, time until exhaustion, or RPE (Allsen et al., 1977; Stephenson et al., 1982b). It is difficult to reconcile these results due to differences in subject population (athletic versus non-athletic), level and duration of exercise, level of $\%VO_2$ max at which the exercise was performed, etc.

Overall, the effect of the menstrual cycle on the cardiorespiratory responses to exercise seem minimal, and probably exert no significant effect on performance of endurance exercise (De Souza et al., 1990; Dombovy et al., 1987; Schoene et al., 1981). Participation in dynamic exercise does magnify some of the hormonal changes that occur across the menstrual cycle. Most notably, the high levels of PRA and ALDO are further increased by exercise in the luteal phase (De Souza et al., 1989; Stephenson et al., 1989). Also, the increase in capillary permeability reported in the luteal phase may be responsible for the more rapid and larger decrease in plasma volume observed during exercise in this phase (Gaebelin & Senay, 1982). In the scientific literature today, there is a paucity of studies addressing the use of oral contraceptives on performance and hormonal changes during exercise. Research from two labs has found that combined oral contraceptive users have an increase in cardiac output and stroke volume during exercise (Lehtovirta et al., 1977; Littler et al., 1974). This may be the result of the increase in plasma volume some studies have reported and could be advantageous during exercise.

It is well known that a major factor contributing to the cessation of exercise by athletes is an increase in body temperature (Nadel, 1988). The physiological responses of women to heat stress and/or exercise, especially relating to thermoregulatory reflexes, has been shown to fluctuate with the menstrual cycle. Basal core body temperature in women is significantly higher during the luteal phase when compared with the follicular phase of the menstrual cycle (Hirata et al., 1986; Horvath & Drinkwater, 1982; Stephenson & Kolka, 1985). Some studies have reported an increase in cutaneous vasodilation and latency to begin thermal sweating (Stephenson & Kolka, 1985), while sweat rate and total sweat loss have been reported to be similar across the cycle (Carpenter & Nunneley, 1988; Pivarnik et al., 1992). There are conflicting reports, however, as to what happens to the core temperature during exercise. It has been found in some studies that the core temperature during exercise is increased at a given work load to a higher temperature in the luteal phase than during the follicular phase (Hirata et al., 1986; Stephenson & Kolka, 1985; Stephenson et al., 1982a). Horvath and Drinkwater (1982) failed to find a difference between phases in core temperature during exercise combined with heat stress. These contradictory findings probably result from the differences in experimental protocol and ambient temperature between the studies. A collection of evidence, including increased basal body temperature, an increased threshold for thermoregulatory sweating and cutaneous vasodilation, the preference of women for a higher skin temperature, and the enhanced vasoconstriction during cooling suggests a resetting of the body's thermal set-point at this time (Bartelink et al., 1990; Cunningham & Cabanac, 1971; Hirata et al., 1986; Kolka & Stephenson, 1989; Stephenson & Kolka, 1985).

Similar to the luteal phase, women using combination oral contraceptives exhibit a shift in the threshold for cutaneous vasodilation to a higher core temperature (Charkoudian & Johnson, 1997). Rogers and Baker (1997) found that oral contraceptive use resulted in higher core temperatures at rest and during exercise. It is believed that the high levels of progesterone in the luteal phase and in oral contraceptives are responsible for resetting the thermoregulatory set point higher. Another possibility is the presence of a relative hypovolemia during the luteal phase (Turner & Fortney, 1984). Whatever the cause, it may be that the increased exercise core temperature in the luteal phase is a fragile phenomenon, easily obscured by the combined demands of exercise and heat stress.

Involuntary dehydration

In the past, few studies have examined the effects of the menstrual cycle on the ability of women to rehydrate satisfactorily following bouts of exercise. Involuntary dehydration is a well-known occurrence in exercising men but remains relatively unstudied in women. It is a phenomenon in which a person drinks to satiety but has still not satisfied the body water deficit (Greenleaf & Sargent, 1965). The degree of dehydration remaining can vary greatly between individuals, with some being termed "reluctant" drinkers (Szlyk et al., 1989b). Involuntary dehydration can delay full rehydration after exercise or water deprivation by several hours. Although the mechanisms underlying involuntary dehydration are not fully understood, they seem to include oropharyngeal inhibition of thirst and the removal of osmotic and volume stimulation.

Rolls et al. (1980) followed the pattern of water intake and sensation of thirst in humans after a 24-hour fluid deprivation. Water deprivation leads to both cellular and extracellular dehydration. The subjects in this study drank more than half of their total 1-hour water intake in the first 2.5 minutes of rehydration and thirst was reduced to pre-deprivation levels. Plasma volume was returned to normal and plasma osmolality were significantly reduced ten minutes following water intake, but this did not fully explain the rapid inhibition of thirst. Subsequent experiments have shown that oropharyngeal stimulation, probably combined with gastric signals, may help to limit intake (Figaro & Mack, 1997; Geelen et al., 1984).

Drinking water alone will lead to a decrease in plasma tonicity and remove the osmotic stimulus for fluid intake (Nose et al., 1988). Maintaining plasma osmolality by ingesting sodium along with water promotes rehydration and reduces the degree of involuntary dehydration remaining (Maughan & Leiper, 1995; Nose et al., 1988). Other factors known to increase intake include a cool water temperature and flavor enhancement (Boulze et al., 1983; Hubbard et al., 1984; Szlyk et al., 1989a). Although ad lib water intake following exercise and degree of involuntary dehydration in women has not been examined across the menstrual cycle, a study has examined the variation in retention of ingestion of a fixed volume. Because of the change in hormones across the cycle, the amount of fluid retained might change between phases. However, no significant changes were observed and the researchers concluded that fluid replacement is not influenced by cycle phase (Maughan et al., 1996). Thus far, no studies have examined water intake or fluid replacement in women using oral contraceptives. One study has found that oral contraceptive users have lower water and mineral output than normal women, both at rest and following exercise (Prashad et al., 1987). Lowered fluid outflow may act to enhance rehydration in women taking oral contraceptives.

Study Proposal

In the scientific literature, there has been a lack of research examining the effects of the menstrual cycle and oral contraceptive use on hydromineral balance following exercise. Endogenous and synthetic ovarian hormones have been shown to influence water and sodium intake in animals, and affect fluid balance in humans. Through both primary and secondary effects, they also modify the concentrations of several of the hormones important in hydromineral balance. Because of the increased RAS and ALDO activity during the luteal phase, the normal exercise response of these hormones may be enhanced at this time. In addition to these changes, some studies have reported a reduction in the threshold for osmotically stimulated thirst along with a slight hypovolemia. Although the variation in OXY levels across the cycle is not distinct, some studies have found that the concentrations are slightly higher in the follicular phase. The combination of all of these changes may result in an increased thirst sensation, voluntary water intake, and sodium palatability during the luteal phase when compared to the first half of the menstrual cycle. Therefore, the degree of involuntary dehydration remaining after rehydration may be reduced in the luteal phase.

Normally, oral contraceptive users have only slightly increased PRA and ANGII levels at rest. However, the RAS and ALDO responses during exercise have not been examined. The increase in plasma renin substrate and angiotensinogen may lead to a magnification in RAS response. Oral contraceptive use is associated with a higher plasma volume level; however, increase in capillary permeability may result in greater plasma volume loss during exercise. These changes suggest that water intake might be enhanced with oral contraceptive use, while the decreased urinary water and sodium output previously noted may promote fluid retention during rehydration. However, the administration of combination oral contraceptives in rats is associated with a decrease in water and sodium intake. This, along with the increase in OXY levels, may reduce sodium palatability in women taking oral contraceptives. Which factors will exert a more powerful effect on water intake and involuntary dehydration remains to be seen.

Aside from the changes in fluid balance with the menstrual cycle and oral contraceptive use, there are also changes in thermoregulatory responses. At rest, women in the luteal phase and those taking oral contraceptives thermoregulate at a higher core temperature. Research thus far has indicated that core temperature during submaximal exercise of up to an hour is not significantly altered. However, during endurance exercise the decrease in cutaneous vasodilation at a given core temperature may result in higher temperatures during the later stages of exercise in the luteal phase and with oral contraceptive use. Changes in thermoregulation may be reflected by increases in perceived exertion ratings with higher core temperatures.

The purpose of this study is to evaluate the effects of the normal monthly variations in several hormones effecting fluid balance on water intake. In addition, the effects of oral contraceptives on fluid intake also will be examined. Other factors that are related to ingestive behavior also will be measured, such as, sensation of thirst, palatability for sodium solution, hormone levels, and degree of involuntary dehydration remaining following ad lib water intake. Another objective of the present proposal is to study performance during endurance exercise on a bicycle ergometer by measuring body temperatures, skin blood flow, fluid loss, and ratings of perceived exertion. These measurements should allow an in-depth comparison between the responses across the menstrual cycle phases. Additionally, these responses will be compared between normally cycling women and those taking oral contraceptives.

RESEARCH DESIGN AND METHODS

Subjects

Seven women, 21-41 years of age, were chosen as subjects for this study, 4 in the non-oral contraceptive group (NOC) and 3 in the oral contraceptive group (OC). Recruiting was done primarily via word-of-mouth, although one subject was recruited from a flyer advertising the study. As part of the screening process the women completed a questionnaire giving both medical and activity history (Appendix A). All the women were healthy and athletic with normal gynecological histories. Three of the women had been using oral contraceptives for more than one year, and continued to take them throughout the study. Due to the difficulty in obtaining fit subjects and time constraints, there was no attempt to control for the brand of oral contraceptive the subjects received. Each woman was allowed to continue taking the oral contraceptive she had been taking before being recruited as a subject. Subject 5 was taking Triphasil-28, a triphasic preparation which consisted of 0.03 mg of ethinyl estradiol and 0.05 mg of levonorgestrel, 0.04 mg of ethinyl estradiol and 0.075 mg of levonorgestrel, and 0.03 mg ethinyl estradiol and 0.125 mg of levonorgestrel. Subjects 1 and 9 were on Ortho Novum 1/35, a monophasic preparation of 0.035 mg ethinyl estradiol and 1 mg norethindrone. The remaining four women were freely cycling, and had not taken oral contraceptives within one year.

Table 2-1 presents a summary of the physical characteristics of the subjects who took part in this study, and the types of exercise in which they would normally participate. The average height, weight, and body mass index (BMI) are similar between the two groups. The BMI is a ratio of body weight to height and is used as an indicator of total body composition (American College of Sports Medicine, 1991). All of the subjects in this study fell within the "healthy" range for this index, 19-25 (Powers & Howley, 1994). The average age of subjects in each group was NOC - 32.25 and OC - 24.3 years. One woman in each group had previously been pregnant, with the pregnancy of the NOC subject being carried to term.

Subjects were chosen for the study based on their pre-study exercise questionnaire and their performance on a submaximal ergometer test. The women gave self-reported estimates of their fitness in the pre-study questionnaire (Appendix A). The question consisted of, "How would you rate your physical fitness (endurance)?", and a Likert-type scale ranging from 1 to 7, with perceptual anchors of 1 = low, 4 = medium, 7 = high. These fitness estimates are also presented in Table 2-1. The women had self-reported fitness levels of medium-high to high and engaged in some form of aerobic exercise several times a week for at least 20-60 minutes (Table 2-2). In previous exercise studies conducted in this laboratory, the resistance a subject would ride at to be working at 60% of their maximum oxygen consumption per minute (VO_2 max) was estimated by heart rate and RPE during the first experimental trial. However, after difficulty with the first subject, (OC-1), who was not very cardiovascularly fit, additional criteria was required of the subsequent subjects. Subjects had to be able to ride an ergometer for 60-90 minutes at a resistance of at least 100 watts (W), and a submaximal ergometer test was used to estimate 60% of their VO_2 max (See Specific Procedures - Submaximal VO_2 test). This study and the procedures used were approved by the Institutional Review Board (study #372-93), and an informed consent was obtained from each subject before they were allowed to participate.

Design

The study consisted of a minimum of eight exercise trials conducted over two complete menstrual cycles. There were four trials during each menstrual cycle; during the menstrual (M), mid-follicular (F), early and late-luteal phases (EL and LL). At least six days elapsed between exercise trials. The physiological data collected during each trial included heart rate, rectal and skin temperatures, skin blood flow, blood samples for hormone analysis, weight change, and urine output. A respiratory gas analyzer determined oxygen consumption (VO_2). Behavioral data collected included RPE, thirst ratings, palatability of sodium solutions, and amount of water intake. A more in depth description of the experimental methods follows in the Specific Procedures section.

Procedure Overview

Each exercise trial consisted of a 90-minute ride on a cycle ergometer, which was adjusted, as determined by the submax VO_2 test, to maintain a stable 60-70% relative VO_2 max. The subjects reported to the lab at 8 am on test days, following 12 hours without food or fluid. Prior to the ride the subjects used visual-analog scales to rate their current sensation of thirst and the palatability of several sodium solution concentrations. A heparin lock catheter was placed in the subject's forearm for the four hours required for completion of each trial, and blood samples for subsequent analysis were obtained via the catheter every 30 minutes. The subject was then fitted with several thermisters for measuring rectal and skin temperatures during the ride. Immediately before the ride a respiratory gas analyzer was used to measure pulmonary gases.

Throughout the experimental trial, the ambient temperature was adjusted to remain between 22-24°C, with a relative humidity of approximately 45%. The ride began with a 10-minute warm-up period after which the subject pedaled at an intensity approximately equal to 60-70% of her estimated VO_2 max for the remainder of the 90 minutes. Skin and rectal temperatures, skin blood flow, and body weight were recorded every three minutes for the first 30 minutes and every 10 minutes for the remainder of the ride. Ratings of perceived exertion were recorded at 10-minute intervals throughout the ride, while severity of thirst was recorded at 30 minute intervals. Pulmonary gas analysis and a blood draw were performed at 30-minute intervals during the ride. The ride was normally terminated after 90 minutes, or when the subject's RPE reached 19, her heart rate reached 80% of her maximum beats per minute, or she could no longer maintain a pedal rate of at least 60 rotations per minute (rpm). No fluid was given during the ride.

After the ride subject sat quietly for 30 minutes to allow the body fluid compartments to stabilize. At the end of the stabilization period the subject gave a thirst rating and performed another sodium palatability test, her body weight was recorded, and a blood sample was taken. For the next two hours the subject was allowed to rehydrate ad lib with distilled water. Ratings of thirst, blood samples, and body weight were recorded at 30-minute intervals during this time. At the end of two hours the subject was asked to void her bladder and her urine output was measured. A final body weight and the total amount of water ingested were also recorded.

Specific Procedures

Pre-study monitoring phase

Prior to the experiments, the subjects monitored and charted their menstrual cycle for at least 1 month, and tried to accurately identify the occurrence of ovulation by taking daily temperatures. This pre-monitoring phase provided data regarding the length of the menstrual cycle, the length of the phases, and whether or not ovulation was occurring. Each subject was asked to record her basal body temperature every morning before rising, then record this on the temperature chart, along with the day of the cycle. During the menstrual phase the subject indicated bleeding by marking an X at the bottom of the chart.

Submaximal VO_2 Test

Prior to the first exercise trial, a brief submaximal VO_2 test was conducted to determine at what intensity, in watts, a subject would ride at to be at 60-70% of her VO_2 max. This was accomplished by a short ergometer test using increasing intensities of workload. Throughout the test expired air was collected for analysis and used to determine the VO_2 at each workload. The subject began pedaling at 50 W for three minutes. The intensity was increased to 75 W for three minutes, and then to 100 W. After this warm-up phase, the intensity was increased by 10 W every 2 minutes until the subject's heart rate was increased to above 80% of her age-adjusted max, the RPE was 18 or above, or she could no longer maintain a pedal rate of at least 60 rpm.

Catheter Placement and Blood Draws

Each subject reported to the lab at 8 am on test days, following a 12 hour fast without food or fluid. A physician's assistant placed an indwelling 20 gauge Novalon heparin-lock IV catheter in the subject's forearm for the four hours required for completion of each trial. One 16 ml blood sample was taken at the time the catheter was inserted and the line was flushed with a 100 units/ml heparin solution to maintain line patency. Subsequent blood samples also were drawn via the catheter. Individual blood samples were approximately 16 ml in volume, with a total of nine blood samples taken per trial. The samples were drawn every 30 minutes during the ride with one blood sample taken before the ride, three blood samples taken during the ride, and five during the recovery period following the ride. Blood collection and storage procedures are described in a later section.

Temperature Measurements

Two non-invasive stainless steel thermister probes (YSI series #409 probes) were used to measure skin temperature. They were taped to the interior aspect of the forearm, one uncovered and the other covered by paraffin to prevent evaporative heat loss. By preventing heat loss blood temperature being delivered to the skin can be estimated. To monitor core body temperature during the ride, a third thermister probe (YSI series #401 probe) was inserted by a nurse into the rectum approximately 15 cm. Ambient room temperature was monitored by a fourth thermister attached to the handlebars of the ergometer. The four thermister probes were plugged into a YSI analogue Tele-

Thermometer, Model 46 TUC. The temperatures were measured every 3 minutes during exercise, to within 0.5°C.

Skin blood flow

Skin blood flow during the ride was measured using a PeriMed PF3 laser Doppler perfusion monitor manufactured by Medex Inc. The probe was attached to the medial aspect of the subject's deltoid prior to the start of exercise and skin blood flow changes were recorded throughout the ride. Laser Doppler flowmetry consists of a probe directing a low energy laser into tissue. Blood cell movement through the tissue results in Doppler-shifted light being reflected back to the probe. This shift is proportional to the flux of blood cells and is quantified by the PeriMed PF3. Blood flow is then reported as laser Doppler blood flow (LDBF) units.

Respiratory gas analysis

A Beckman Metabolic Measurement Cart was used to measure absolute and relative oxygen consumption (VO_2). The Beckman MMC uses the carbon dioxide rebreathing technique to determine VO_2. Each subject wore headgear fitted with the appropriate mouthpiece, nose clip, respiratory hoses and valves. The subject was only required to wear these accessories during the 5 minutes it took to complete each test. During the test, inspired air is obtained from a bag filled with a known concentration of CO_2 (8 or 13%) and expired air is routed into the MMC for analysis. The computer calculates the volume of oxygen inspired and expired, and the volume of carbon dioxide produced. From these results the absolute VO_2 can be computed. By adjusting this figure for the subject's body size, the relative VO_2 can be estimated. The respiratory gas analysis test was conducted immediately before the ride and at 30 minute intervals during an exercise trial.

Body Weight

Sweat rate was estimated by weight loss during the ride. The ergometer rested on Datex WM 104a bed scales, accurate to < 10 g, which allowed for the continuous recording of body weight throughout the ride. Body weight was recorded every 3 minutes for the first 30 minutes of the ride, and every 10 minutes for the remainder of the exercise trial. Sweat rate was calculated from the change in body weight divided by the number of minutes over which the change occurred. After the ride, weight was measured every 30 minutes, either by having the subject sit on the ergometer or weigh on a Health-O-Meter medical scale. The body weight recorded during the exercise trial was compared to the subject's initial weight recorded at the start of the ride. This was used to calculate percent of body weight change, [(end rehydration body weight / begin body weight)*100-100], and percent of involuntary dehydration remaining, [(end rehydration body weight / end ride body weight)*100].

Ride Section

Each exercise trial was divided into three sections: ride (RD), rest (RST), and rehydration (RHYD). The RD section consisted of an ergometer ride for a maximum of 90 minutes. Each subject began the ride with a 9-minute warm-up period. The subject began at an intensity of 50 W, which was increased every three minutes to 75, 100, and 110 W. The intensity was then increased by 10 W every 3 minutes until the subject was at the intensity she was to ride at, as determined by the submaximal VO_2 test. Throughout the ride the subject was asked to maintain a pedal rate of between 60-80 rpm.

Rest and Rehydration Sections

The RST section immediately followed the RD section. During this section the subjects sat quietly for 30 minutes to allow the exercise/thermoregulatory-induced vascular changes to return to normal and the body fluid compartments to stabilize. At the end of the RST section the subjects gave a thirst rating, performed another solution palatability test, a blood sample was taken, and the subject's weight was recorded.

For the next two hours the subjects were in the RHYD section and allowed to rehydrate ad lib with H_2O. The water was distilled water, chilled to 5-10°C. It was placed in an insulated 1000 ml water bottle with a straw. Ratings of level of thirst were recorded at 30-minute intervals. Blood samples were taken every 30 minutes, total body weight was recorded, and the amount of urine output was recorded throughout the RHYD section.

Blood collection and analysis

The nine blood samples that were taken during each trial were drawn through 21 x 3/4 Butterfly needle and tubing infusion sets and into 35 ml syringes. First, enough fluid was drawn out through the tubing to remove the heparin. A clean infusion set and syringe was used to draw the 16 ml blood sample. The catheter was then flushed with approximately 10 ml of the heparin solution to prevent clotting.

The blood collected was then aliquoted into chilled tubes. Five ml were placed in a red-top vacutainer and 2 ml in a lavender top vacutainer. These samples were refrigerated and sent to Labcorp Clinical Laboratory for a chemistry panel that includes glucose, sodium, and potassium levels, and a complete blood count (CBC). The CBC allows for calculation of percent changes in blood and plasma volume as described by Dill and Costill (1974).

The remainder of the blood was divided into a 3 ml tube for serum, a 3 ml tube with 10 μl EDTA for plasma, and a 2 ml tube with EDTA and 50 μl of bestatin. The EDTA was a solution of 100 mg/ml distilled water, and the bestatin was a 0.5 mM solution in 5% methanol and 95% saline. Bestatin prevents the breakdown of angiotensin I into ANGII. These tubes were centrifuged at 3000 rpm for 15 minutes, the plasma or serum drawn off, and the appropriate amount aliquoted into microtubes for the hormonal assays. The samples were frozen at -75°C until the assay was completed.

The samples were analyzed for blood levels of PRA, ANGII, ALDO, and progesterone by radioimmunoassy (RIA). Samples also were collected to measure ADH and OXY. However, high temperatures in the centrifuge ruined the samples. Plasma renin activity was measured by the Angiotensin I (I^{125}) RIA kit (NEA 104) from DuPont NEN of Boston, MA. Angiotensin II was measured by a double-antibody RIA kit (RK-A22) from American Laboratory Products Co. (ALPCO) of Windham, NH. The progesterone (07-270102) coated tube kit was obtained from ICN Pharmaceuticals, Inc. of Costa Mesa, CA. Aldosterone was assayed by the Coat-a-Count kit (TKAL1) from Diagnostic Products Corporation of Los Angeles, CA.

Ratings Scales

Ratings of perceived exertion

Borg's 15 point RPE scale was used throughout the exercise trial (Figure 2-1). The scale consists of numbers from 6-20. Beginning with 7, every other number was anchored with a written statement such as ' very, very light'. At 10 minute intervals throughout the ride, the subject was asked to indicate her perceived exertion by giving a number from the scale that corresponds to how hard she felt she was working at that time.

Ratings of thirst

During each exercise trial ratings of thirst were obtained every 30 minutes, for a total of 9 responses, using a visual analogue scale based on Rolls et al. (1980). The scale consisted of the question "How thirsty do you feel now", below which is a 10 cm line with the extremes "Very thirsty" and "Not at all thirsty" at either end (Appendix B). The subject read the statement then placed a vertical mark along the continuum at the point they felt best described their sensation of thirst.

Ratings of sodium solution

Following the catheter placement and 30 minutes after completion of exercise, the subject performed a "swig and spit" test on five sodium solution concentrations, 0.01, 0.03, 0.06, 0.1, and 0.15M. The order of the concentrations was randomized for each test. Five small disposal cups were labeled A through E in a single-blind fashion. The subject tasted each sample, beginning with A, and spat it into a collection container. The subject's palatability rating for each sample was then assessed using a visual-analog scale (Appendix C). The scale consisted of the question "How would you rate the taste of this solution?", below which was a 10 cm line anchored by the extremes "Dislike extremely" and "Like extremely". The subject then placed a vertical mark at the point she felt best described the sodium palatability. After each sample the subject rinsed her mouth with distilled water, spat the water into the collection container, and tasted the next sample until all five had been tasted.

Statistical Analysis

The results of this study are presented as adjusted means. The physiological, water intake, and dehydration data were analyzed by a univariate repeated ANOVA (SAS statistical software package, Cary, NC). Because of the complicated design of the experiment, a SAS procedure known as PROC MIXED was used to analyze the data. PROC MIXED is a flexible procedure which can accommodate multiple random effects of varying types, allows several factors to be repeated at the same time, can handle an unbalanced design, and adjust for missing data. When statistical significance was reached, Tukey's HSD procedure was used as a *post-hoc* test to identify the differences between the least square means.

The subjective ratings data (RPE, thirst and solution ratings) were analyzed as discussed above, as opposed to some type of nonparametric procedure, for several reasons. It is preferable to analyze nonparametric data using parametric statistics if the assumptions for parametric procedures can be met (Norusis, 1991). These assumptions include the data having a normal probability distribution with equal variances and being based on interval measurement (Agresti & Finlay, 1986). The Borg RPE scale is a category scale and could result in difficulties when making interindividual comparisons

(Borg, 1982). However, the scale was designed to increase linearly with exercise intensity and it has been demonstrated to have high correlations with heart rate and relative VO_2 (Dunbar et al., 1992). The thirst and solution ratings data were obtained using interval scales. The ratings data distributions were tested for normal probability distributions. All the data passed the normality test except for the data from the 0.06, 0.10, and 0.15 M solutions for the OC group. Unfortunately, there were no appropriate nonparametric statistics that could be used due to the complicated experimental design. Therefore, the ratings data were analyzed with a univariate repeated ANOVA, and the non-normal distributions will be discussed in the results when appropriate.

Several ANOVA comparisons were made for each response measured. These included within group comparisons across time or ride section and across phase. For the NOC and OC groups, separate one-way ANOVAs were conducted with time, ride section, or phase as the repeated measures. Measures that were recorded during exercise only (e.g. temperatures, RPE) were compared across time while responses obtained throughout the exercise trial (e.g. blood measures, thirst) were compared across ride section. Between group ANOVAs were conducted on the overall responses of each group without consideration of time, ride section, or phase. Three additional one-way repeated ANOVAs compared the groups while blocking for time, ride section, and phase. The baseline (Time 0) response was excluded for all of the phase comparisons to remove the expected change in magnitude due to exercise. This added variance might have obscured any changes across phase.

Table 2-1. Physical Characteristics of Subjects.

Subject #	Group	Age	Weight (kg)	Height (cm)	BMI	Fitness Level
OC-1	OC	21	52.82	160.02	20.63	5
OC-5	OC	25	64.53	171.45	21.96	6
OC-9	OC	27	63.47	162.56	24.00	6
Average	OC	24.3	60.27	164.68	22.20	5.7
NOC-2	NOC	40	57.34	165.10	21.03	7
NOC-3	NOC	24	64.88	163.19	24.36	6
NOC-8	NOC	33	58.64	167.64	20.88	7
NOC-11	NOC	32	68.12	167.64	24.25	6
Average	NOC	32.25	62.24	165.89	22.63	6.5

Note: OC = oral contraceptive group; NOC = non-oral contraceptive group. Weight was calculated by averaging the pre-ride weight of each subject across the eight rides. Body Mass Index (BMI) was calculated by WT (kg)/HT^2 (m). Fitness level (self-reported) was from a scale ranging from 1 (low) to 7 (high).

Table 2-2. Physical Activity Engaged in More Than 3 Times Per Week.

Subject #	Running	Cycling	Other (aerobics, walking)
OC-1			20 - 40 min.
OC-5	> 60 min.		
OC-9		> 60 min.	
NOC-2	> 60 min.		
NOC-3	20 - 40 min.		
NOC-8	> 60 min.		
NO-11	20 - 40 min.	> 60 min.	40 - 60 min.

Note: Predominant aerobic activity of each subject and the average time they engaged in that exercise per session.

6	
7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

Figure 2-1. Borg's Rating of Perceived Exertion Scale.

RESULTS

Problems Generated During the Study and Analysis

A summary of the results obtained from the statistical analysis is presented in Table 3-1. During the collection of data and statistical analysis several problems related to the level of the subjects' fitness and timing of the experiments became apparent. These problems are discussed in greater detail in the following sections.

Subject number and characteristics

Due to the difficulty in obtaining fit subjects and the length of time required to complete all the exercise trials, only a small number of subjects were included in this study. This led to a lack of statistical power during the analysis and a difficulty in finding significant differences. Another problem related to the small subject number in this study is the imbalance in subject age and race between the groups. Only one African-American woman volunteered as a subject and was subsequently included in the NOC group. An examination of the predicted values and residuals for each parameter measured did not show any evidence of an ethnicity effect. With regards to the ages of the subjects', there was almost no overlap between the groups (Table 2-1). Although there is a decrease in thirst sensation and water intake in the elderly (>65 years), the oldest subject in this study was only 40 years old. A correlation analysis in which age was tested with thirst rating and water intake did not find any significant effect of age.

Subject fitness

The submaximal test used to estimate the workload for each subject was discussed in Chapter 2. A *post hoc* estimation of the subjects' VO_2 max was used to verify the %VO_2 max each subject rode at during the study. This calculation is based on the linear relationship between VO_2 and heart rate. Heart rates at three different workloads during the submaximal ergometer test were plotted, and a best-fit line drawn through these points. This line was extrapolated to the subject's age-adjusted maximum heart rate (220-age), and this point was used to estimate the VO_2 max (Figure 3-1). These post-study calculations suggest that the submaximal test protocol underestimated the workload of two of the subjects in the NOC group (Table 3-2). Five of the subjects rode at an intensity equal to approximately 60-70% of their VO_2 max. However, NOC-2 and NOC-8 were much more cardiovascularly fit than the other subjects. The calculations suggest that they may have ridden at only 40-45% of their VO_2 max. Subjects working at a lower relative intensity may have lower body temperatures, SBF, and smaller plasma volume changes than subjects' working at higher relative intensities. An examination of the highly trained subjects' mean values for these parameters did not indicate any values inconsistent with the other subjects in the NOC group.

Canceled or incomplete rides and ride chronology

On several occurrences, a subject became ill before an exercise trial was begun or during the trial. The incidents where one subject felt ill before the ride occurred after the catheter placement took more than one needle stick. It appeared to be a stress reaction and the subject felt better within a few minutes. The exercise trial for that day was canceled and rescheduled for the next menstrual cycle.

Three of the subjects stopped exercising before 90 minutes on at least one of their 8 rides. In all but three of these cases, the subjects had been exercising for at least 60 minutes and the study was completed for that day. For the trials where the subjects quit before 60 minutes of exercise, that ride was not counted and the ride for that phase was rescheduled for the next menstrual cycle.

In addition to the problems discussed above, there were problems in scheduling the exercise trials. Most subjects were not able to ride once a week for 8 straight weeks, so a ride for a particular phase would be obtained in a subsequent menstrual cycle. Table 3-3 shows the exercise trial chronology for each subject. For example, subject NOC-2 began the study with a trial during the F phase and completed all 8 trials in a row, with the phase order being F, EL, LL, M, F, EL, LL, M. Subject NOC-3 also began the study during the F phase, but due to scheduling problems it took a total of 6 cycles (25 cycle phases) to obtain all eight trials, with the phase order being F, F, EL, M, EL, LL, LL, M. Because each phase was being considered as a separate 'treatment', it did not matter from which menstrual cycle it was obtained, so chronology was not considered to be a problem.

Verification of ovulation

As discussed in the previous chapter, basal body temperatures were taken daily in an attempt to predict ovulation so that the luteal rides (EL and LL) could be scheduled for post-ovulation. Following the completion of data collection, the progesterone levels on each ride-day were measured to verify the cycle phase. In this study, the L phase was defined by progesterone levels of >3 ng/ml. When this criterion was applied, three of the experiments with the NOC subjects had to be reassigned *post hoc* to the phase in which they actually took place. For example, if the trial had been scheduled as a F trial, but an ovulation had already taken place, the trial was re-labeled as an EL trial. If the trial was scheduled as an EL trial, but an ovulation had not occurred yet, it was relabeled as an F trial. Because the verification and reassignment of phases was done after completion of the data collection, it led to an unequal *n* per phase (i.e. 3 F trials and 1 EL trial for NOC-8).

Physiological Data

Core Temperature

The ANOVA indicated a significant difference across time for both groups at p< .001 (Figure 3-2). The *post hoc* test revealed that core body temperature at 30, 60, and 90 minutes of exercise was significantly greater from baseline in both groups. Additionally, the core temperature in the NOC group at 90 minutes was significantly greater than at 30 minutes of exercise. An ANOVA comparing the core temperatures from 30 to 90 minutes during the ride indicated that there was no significant difference across phase in either group (Figure 3-3). Between group comparison of the core body temperatures during the ride and across cycle phase also failed to find a significant difference.

Uncovered Skin Temperature

The ANOVA indicated a significant increase in uncovered skin temperature (USK) during the ride in the NOC group (p< .01). There was no increase in USK in the

OC group. *Post hoc* analysis on the NOC group indicated that the USK at 30, 60, and 90 minutes was significantly increased from the baseline temperature ($p < .05$). The USK during exercise is shown for both groups in Figure 3-4. There was no significant difference in USK between cycle phases in either group (Figure 3-5). Although the mean USK for the NOC group was consistently greater than that of the OC, both during the ride and across cycle phase, the ANOVA did not find a significant difference between the groups.

Covered Skin Temperature

The ANOVA for covered skin temperatures (CSK) showed significant effects of time for both the NOC and OC groups at $p < .0001$ (Figure 3-6). *Post hoc* results show that the CSK for both groups was significant from baseline at 30 minutes of exercise through 90 minutes ($p < .001$). There were no significant differences across menstrual phases for either group (Figure 3-7).

There were no significant differences found in any of the comparisons between the groups, although the NOC had higher CSK throughout the ride and across cycle phase (Figures 3-6 and 3-7).

Laser Doppler Blood Flow

Laser Doppler blood flow was significantly increased during exercise in both groups (NOC $p < .05$, OC $p < .001$), Figure 3-8. *Post hoc* analysis showed the LDBF significantly increased from baseline at 30 and 60 minutes in both groups (NOC $p < .05$, OC $p < .005$), and from baseline to 90 minutes in the OC group ($p < .005$). Laser Doppler blood flow changes across menstrual phase were significant in the NOC group ($p < .05$)

but not the OC group (Figure 3-9). Results from the *post hoc* analysis indicated a significant difference between the F and EL phases in the NOC group, p < .05. Although no overall difference was found between the groups or when the groups were compared across time by ANOVA, there was a significant difference between the groups during the EL phase, p< .05 (Figure 3-9).

Body Weight

The average change in total body weight after the RD section was -1.2% for the NOC group and -1.3% for the OC group (Figure 3-10). Following the RHYD section the weight loss had been decreased in both groups, although only the NOC group approached significance (NOC = p< .06). The average weight change from baseline following rehydration was NOC= -0.52% and OC= -0.83%. There was no significant difference within either group between phases of the menstrual cycle or in any of the comparisons between the groups (Figure 3-11).

Blood analysis

Plasma Volume

The average percent changes from baseline in plasma volume for both groups are presented in Figure 3-12. Subjects in the NOC group lost 5.41% plasma volume during the RD, while the OC subjects averaged losses of 7.63%. An ANOVA indicated a significant change in plasma volume across trial section for both groups (NOC = p< .001; OC = .001). *Post hoc* results show that the plasma volume of both groups during the RST and RHYD sections was significantly greater than the RD plasma volume (NOC = p< .001; OC = p< .005,). The change in plasma volume from RST to RHYD was also significant in the OC group ($p < .05$). During the RST section, subjects in both groups regained a significant amount of plasma volume, such that they were slightly volume-expanded. The plasma volume increased slightly more during the RHYD section. The changes in plasma volume across cycle phase were not significant for either group, nor were comparisons between the groups significant (Figure 3-13).

Plasma Renin Activity

The mean PRA levels across the experimental trial are shown in Figure 3-14 for both groups. ANOVA revealed significant differences between experimental sections for both groups at the $p < .05$ level. *Post hoc* tests showed that PRA levels in both groups were significantly increased from baseline by exercise (NOC $p < .005$; OC $p < .01$). Plasma renin activity between the RD and RHYD sections also was significantly different, $p < .05$. For the OC group, PRA levels during the RST section approached significance when compared with levels during the RHYD section ($p < .06$). The PRA levels after RHYD were not significantly different from the baseline levels in either group.

The NOC group levels were significantly different across phase ($p < .05$) for both the RD and RHYD sections, while OC group levels were not (Figure 3-15). Post hoc tests showed that PRA levels during the RD section of the LL phase were significantly different from the menstrual and follicular phases ($p < .05$). Although PRA levels during the RHYD section appeared to be elevated in the LL cycle phase above the other three phases, the *post hoc* analysis did not indicate a significant difference.

The baseline mean PRA levels for the NOC and OC groups were 1.4 ng/ml (SE .24) and 1.7 ng/ml (SE .28), respectively. Comparison of the PRA levels between groups found that the OC group had higher levels overall, although the p value only reached .09. The groups were then compared by ANOVA across time and then by phase. The OC group had significantly higher PRA levels across RST and RHYD sections of the experimental trial ($p < .05$). They also had significantly higher levels during the M and F phases in the RD section ($p < .05$) and during the F and EL phases in the RHYD section ($p < .05$).

Angiotensin II

When analyzed by an ANOVA, the ANGII levels changed significantly across the experimental trial in the NOC group ($p < .05$), while the OC group levels did not reach significance ($p < .07$). *Post hoc* tests found that ANGII levels were significantly increased from baseline by exercise (NOC $p < .01$; OC $p < .05$). Angiotensin II levels during the RD section were significantly greater than those during the RHYD section ($p < .05$). The changes for both groups across section of experiment are shown in Figure 3-16. The NOC group ANGII levels changed significantly across phase ($p < .05$) and the OC group did not (Figure 3-17). Similar to the PRA results, *post hoc* analysis of the NOC group indicated that the ANGII levels during the RD section in the LL phase were significantly higher than levels during the M and F phases ($p < .05$). During the RHYD section, the p values for the LL phase compared to the M and F phases only reached .09 and .06, respectively. The ANGII levels after RHYD were not significantly different from the baseline levels in either group.

The baseline ANGII levels for the NOC and OC groups were 35.3 pg/ml (SE 6.2) and 28.4 pg/ml (SE 7.2), respectively. An ANOVA comparing the overall ANGII levels between the two groups did not find a significant difference. Although the OC group's levels were higher than the NOC group during the experimental trial and across most of the cycle phases, significance was reached only during the RHYD section of the F phase ($p < .05$).

Aldosterone

Both groups showed significant changes in ALDO levels across the experimental trial (NOC $p < .001$; OC $p < .05$) when analyzed by ANOVA (Figure 3-18). *Post hoc* analysis showed that exercise significantly increased ALDO levels from baseline in both groups (NOC $p < .001$; OC $p < .05$). For both the NOC and OC groups the ALDO levels during the RHYD sections were significantly lower than either the RD or RST sections (NOC $p < .001$; OC $p < .05$). The ALDO levels after RHYD were not significantly different from the baseline levels in either group. As seen in the PRA and ANGII levels, the NOC group showed significant changes in ALDO across phase ($p < .05$). Post hoc analysis showed both the RD and RHYD sections had elevated ALDO levels during the LL phase when compared to the M and F phases ($p < .05$). The OC group's ALDO levels did not change across cycle phase (Figure 3-19).

The baseline ALDO level for the NOC group was 189 pg/ml (SE 40), while the OC group baseline was 97 pg/ml (SE 46). An ANOVA comparing the overall levels of the two groups found no significant difference in ALDO levels. ANOVAs were then conducted comparing the groups across trial section and by phase. Even though the mean

ALDO level for the NOC group was higher than the OC group during the LL phase (Figure 3-19), there were no significant differences in either comparison.

Behavioral data

Water intake

To adjust for the body sizes of the subjects, each individual's total 2-hour water intake was expressed as milliliters of water ingested per kiligram of body weight. Water intake data were analyzed by ANOVA within groups across phase and between groups (Figure 3-20). The mean water intake for the NOC group was 14.9 ml/kg and for the OC group was 11.7 ml/kg (Figure 3-21). There were no significant differences in any of the ANOVA comparisons.

Involuntary Dehydration

The percentage of dehydration remaining after RHYD, involuntary dehydration, was analyzed by ANOVA across phases and between groups (Figure 3-22). The mean percentage of dehydration remaining was 28% for the NOC group and 53% for the OC group (Figure 3-23). There were no significant differences in these comparisons.

Ratings of Perceived Exertion

As shown in Figure 3-24, the RPE increased significantly during the ride in both the NOC and OC group (NOC $p< .0005$; OC $p< .01$). In both groups, *post hoc* analysis showed that RPE increased significantly from baseline at 30, 60, and 90 minutes (NOC $p< .005$; OC $p< .05$). There was no significant change in RPE across cycle phase in either group (Figure 3-25). There was no significant difference overall between groups, or when compared by time and by phase.

Thirst Ratings

The change in ratings of perceived thirst for both groups across trial section is shown in Figure 3-26. The mean baseline thirst ratings were 6.07 and 6.49 for the NOC and OC groups, respectively. The ratings for both groups increased during the RD section, continued to increase slightly during the RST section, and decreased significantly during the RHYD section. Data analysis by ANOVA found both groups had significant changes across ride section ($p< .005$). *Post hoc* analysis showed the RHYD was significantly decreased from the RD and RST sections in both groups (NOC $p< .0001$; OC $p< .0005$). Analysis comparing the thirst ratings within groups across phase and between the groups did not reveal any significant differences (Figure 3-27).

Solution Palatability Distributions

The OC group palatability data for the 0.06 - 0.15 M sodium solutions demonstrated a bimodal distribution when tested for normality. The sodium palatability of subject OC-9 was higher than that of the other two OC subjects. This raised the groups' mean palatability ratings and made it appear stable across the 5 sodium concentrations. When the median palatability ratings of the two groups were compared, they were almost identical. After statistical consultation, it was decided that experimenting with data transformation would probably not result in significantly different results.

0.01 M Solution Ratings

Solution palatability ratings for the 0.01 M sodium solution before and after exercise are shown in Figure 3-28 while the palatability ratings across the menstrual phase is shown in Figure 3-29. There were no significant differences within either group across ride section or due to phase of cycle.

An ANOVA comparing the overall palatability ratings between the groups found the NOC group had a significantly higher 0.01 M solution palatability rating when compared to the OC group ($p < .05$ level). There was also a significant difference when the groups were compared by ride section (PRERD $p < .05$; POSTRD $p < .01$). The NOC group 0.01 M solution palatability ratings were significantly higher than the OC group during M, EL, and LL cycle phases ($p < .05$).

0.03 M Solution Ratings

There were no significant changes across the ride sections or across phases within either group (Figures 3-30 and 3-31). The NOC group palatability ratings for the 0.03 M solution were higher than the OC group palatability ratings, but none of the between group comparisons reached significance.

0.06 M Solution Ratings

Data analysis did not reveal any significant differences in 0.06 M solution ratings, either within groups or between groups (Figures 3-32 and 3-33).

0.10 M Solution Ratings

Neither group showed a significant change from PRERD to POSTRD (Figure 3-34). However, as shown in Figure 3-35, both groups exhibited changes in 0.10 M solution palatability ratings across phase, with the NOC group changes reaching significance (NOC $p < .05$). *Post hoc* analysis showed that the 0.10 M solution palatability ratings during the M phase were significantly greater when compared to the F and LL phases (p< .05). The differences in solution palatability ratings between the groups did not reach significance, but the mean palatability ratings of the OC group for the 0.10 M solution appeared to be slightly greater than the NOC group's palatability ratings.

0.15 M Solution Ratings

The results of the 0.15 M solution palatability ratings for both groups are presented in Figure 3-36 and Figure 3-37. None of the comparisons within groups or between groups was significant. However, like the 0.10 M solution palatability ratings, the OC group appeared to have a slightly higher mean ratings than the NOC group for the 0.15 M solution but did not reach significance.

Summary of Solution Palatability Ratings

Each group's mean solution palatability rating for the five sodium concentrations is presented in Figure 3-38. The NOC group palatability ratings ranged from 7.57 (0.01 M) to 3.07 (0.15 M), with the palatability ratings decreasing as the sodium concentration increased. The OC group had comparatively stable ratings across the increasing concentrations, with ratings ranging from 4.70 (0.01 M) to 4.04 (0.15 M). The NOC group's sodium palatability ratings significantly decreased as the sodium concentrations increased, while the OC group's palatability ratings did not change across concentration (NOC p< .05).

Table 3-1. Summary of Results.

Response Measured	Comparison Made			
	During Ride	Across Ridesection	Across Phase	Between Groups
Core Temperature	**NOC, OC=.001**	NA	NS	NS
Uncov. Skin Temperature	**NOC = .01** OC = NS	NA	NS	NS
Cov. Skin Temperature	**NOC, OC=.001**	NA	NS	NS
Laser Doppler Blood Flow	**NOC = .05** **OC = .001**	NA	**NOC=.05** OC=NS	NS
Body Weight	NA	NOC = .06 OC = NS	NS	NS
Plasma Volume	NA	NOC, **OC=.001**	NS	NS
Plasma Renin Activity	NA	**NOC, OC = .05**	**NOC=.05** OC = .07	.09
Angiotensin II	NA	**NOC = .05** OC = .07	**NOC=.05** OC = NS	NS
Aldosterone	NA	**NOC=.05** **OC=.001**	**NOC=.05** OC=NS	NS
Water Intake	NA	NA	NS	NS
Invol. Dehydration	NA	NA	NS	NS
RPE	**NOC, OC=.01**	NA	NS	NS
Thirst Rating	NA	**NOC, OC=.01**	NOC; OC =NS	NS
Solution Palatability (0.01 M)	NA	NS	NS	**.05**
Solution Palatability (0.03 M)	NA	NS	NS	NS
Solution Palatability (0.06 M)	NA	NS	NS	NS
Solution Palatability (0.1 M)	NA	NS	**NOC = .05** OC = NS	NS
Solution Palatability (0.15 M)	NA	NS	NS	NS

Note: Statistically significant p values are shown in bold type, NS = not significant, and NA = not applicable. Values that approached significance are also shown. The between groups comparison shown is the overall difference, without blocking for ride section or phase.

Table 3-2. Subject VO_2, relative and absolute.

Subject	Relative VO_2 max (ml/min.*kg)	Actual %VO_2 max During Ride	Watts Cycled at During Ride	# of Rides Stopped Prior to 90 minutes
OC-1	31.42	67 %	70	4
OC-5	42.87	58 %	120	1
OC-9	42.78	58 %	110	0
NOC-2	54.24	44 %	110	0
NOC-3	41.18	57 %	115	3
NOC-8	67.7	40 %	125	0
NOC-11	39.53	69 %	120	0

Note: *Post hoc* estimation of each subject's maximum oxygen consumption (VO_2 max) expressed relative to body weight, in ml • kg^{-1} • min^{-1}. Also included is the approximate percent of VO_2 max at which they exercised during the study and the absolute intensity in watts at which each subject cycled.

Table 3-3. Chronology of Exercise Trials.

Subject	Phase of Menstrual Cycle																													
	M	F	E	L	M	F	E	L	M	F	E	L	M	F	E	L	M	F	E	L	M	F	E	L	M	F	E	L	M	F
OC-1	O	X	X	X	X	X	X	X	X																					
OC-5	X	X	X	X	X	O	O	O	O	X	X	X																		
OC-9	O	X	X	O	X	X	O	O	O	O	O	O	O	O	O	X	O	O	O	X	X	O	X							
NOC-2	O	X	**X**	X	X	**X**	X	X	X																					
NOC-3	O	X	O	O	O	X	**X**	O	X	O	X	X	O	O	O	X	O	O	O	O	O	O	O	O	X					
NOC-8	O	O	**X**	X	O	X	X	X	X	O	O	O	X	X																
NOC-11	X	O	O	O	X	O	O	O	O	O	X	X	O	O	O	X	O	O	O	O	O	X	X	O	O	O	O	O	O	X

Note: Table 3-3 shows the order in which the exercise trials were completed for each subject. The table begins with the first cycle in which the subject began the study, and ends with the last exercise trial. The phases of the menstrual cycle are abbreviated as M - menstrual, F - follicular, E - early luteal, and L - late luteal. In the body of the table a completed ride is noted by an X, and a phase in which an exercise trial was not scheduled or completed is noted by an O. Rides that were reassigned *post hoc,* from either F to EL or EL to F, are noted by an **X.**

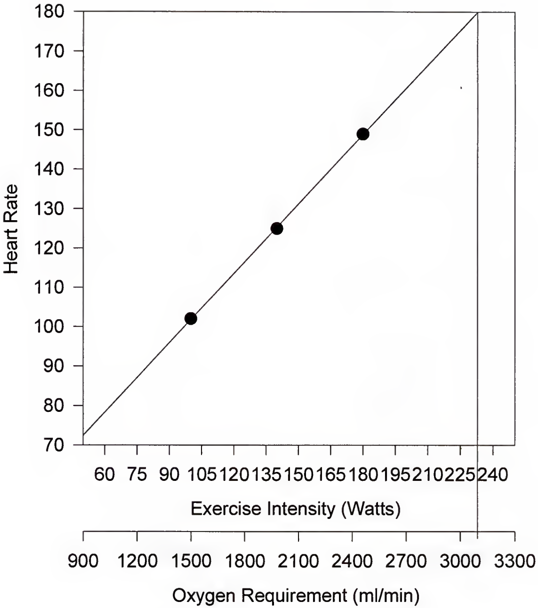

Figure 3-1. NOC-2 submaximal exercise results. Example of the graphs based on submaximal exercise test results. These were used to estimate the VO_2 max of each subject.

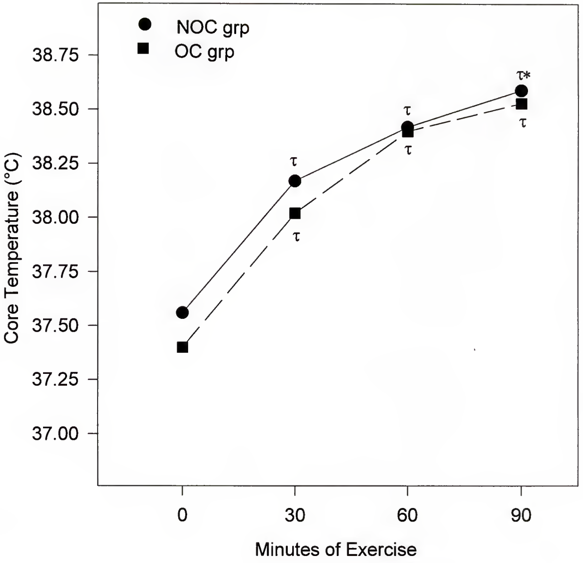

Figure 3-2. Core temperature during exercise. Mean temperatures for both the NOC and OC groups are shown. ANOVA indicated an overall significant difference in both groups across time ($p< .001$). τ - Significantly different from Time 0 (NOC $p< .01$; OC $p< .05$). * - Significantly different from Time 30 ($p< .05$)

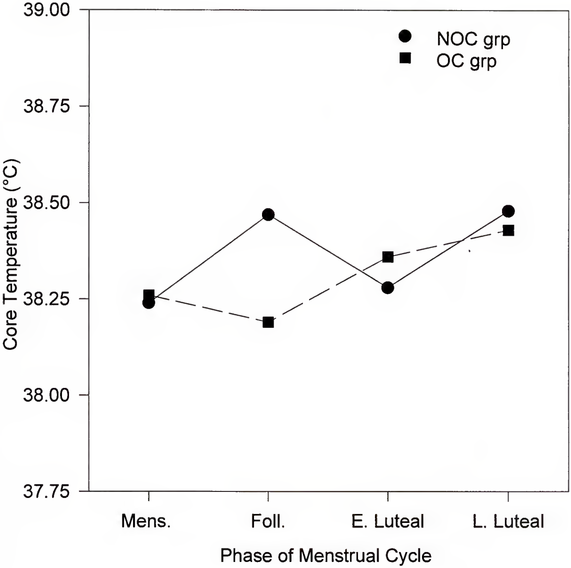

Figure 3-3. Core temperature across phases. Temperatures for both the NOC and OC groups are shown. Values were obtained by averaging temperatures from Time 30 - Time 90 during exercise (means). ANOVA did not indicate any significant differences across phase, either within subjects or between groups.

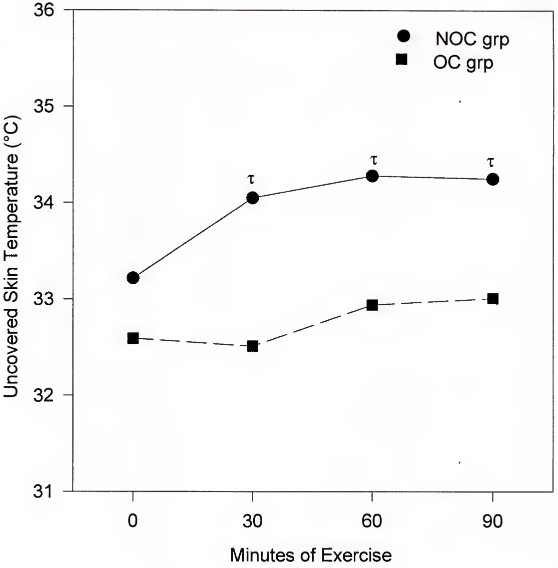

Figure 3-4. Uncovered skin temperature during exercise. Mean temperatures for both the NOC and OC groups are shown. ANOVA indicated an overall significant difference in the NOC group across time ($p < .01$). τ - Significantly different from Time 0 (NOC $p < .05$).

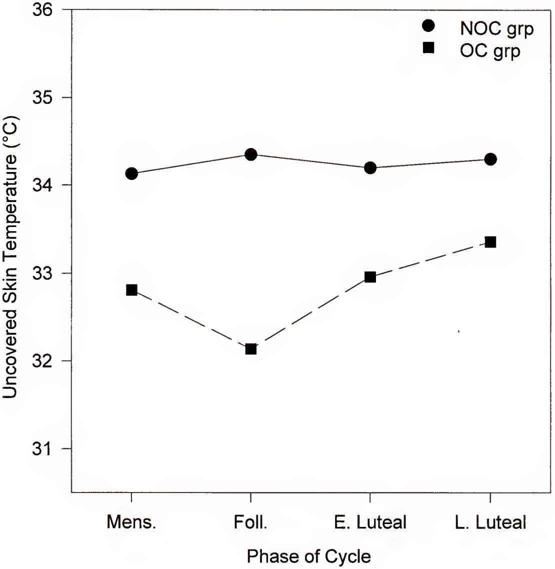

Figure 3-5. Uncovered skin temperature across phases. Temperatures for both the NOC and OC subjects are shown. Values were obtained by averaging temperatures from Time 30 - Time 90 during exercise (means). ANOVA did not indicate any significant differences across phase, either within subjects or between groups.

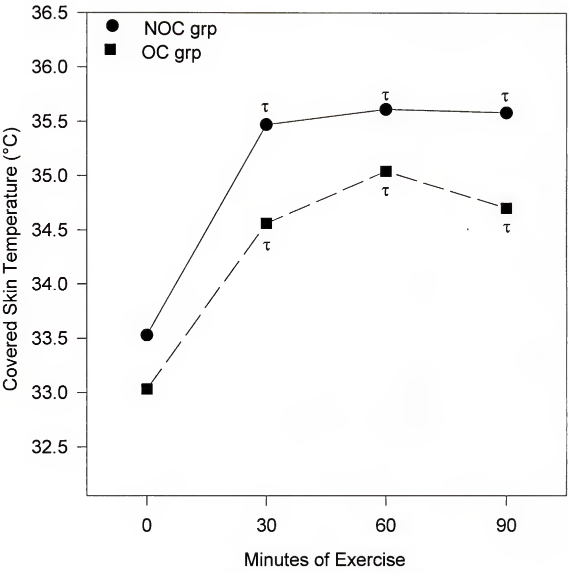

Figure 3-6. Covered skin temperature during exercise. Mean temperatures for both the NOC and OC groups are shown. ANOVA indicated an overall significant difference in both groups across time ($p < .0001$). τ - Significantly different from Time 0 (NOC $p < .001$; OC $p < .001$).

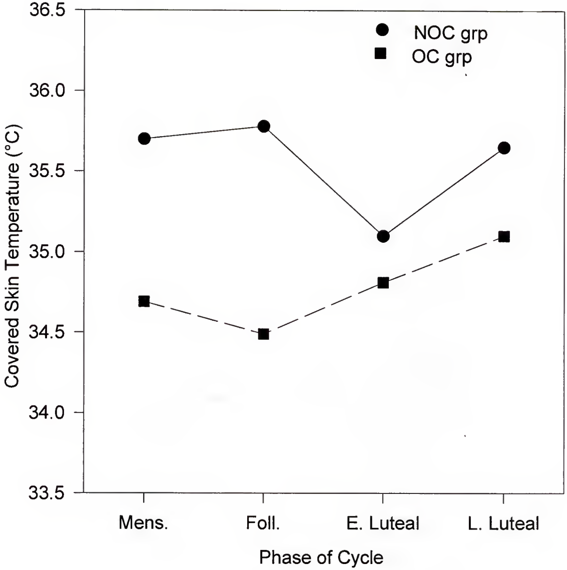

Figure 3-7. Covered skin temperature across phases. Temperatures for both the NOC and OC groups are shown. Values were obtained by averaging temperatures from Time 30 - Time 90 during exercise (means). ANOVA did not indicate any significant differences across phase, either within subjects or between groups.

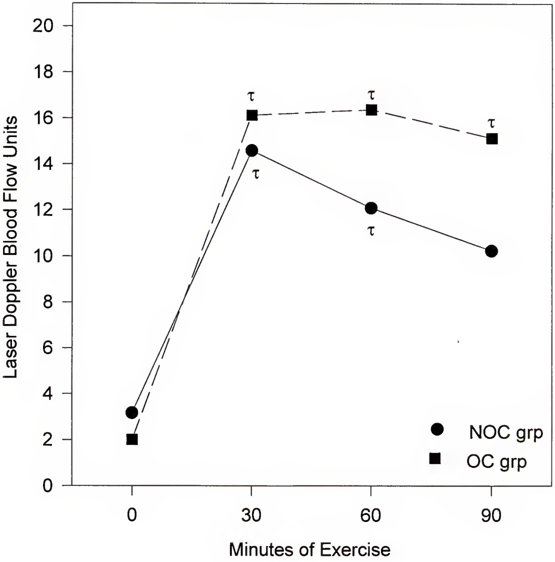

Figure 3-8. Skin blood flow during exercise. Mean Laser Doppler blood flow during exercise in the NOC and OC groups. ANOVA indicated an overall significant difference in both groups across time (NOC $p < .05$; OC $p < .001$). τ - Significantly different from Time 0 (NOC $p < .05$; OC $p < .005$).

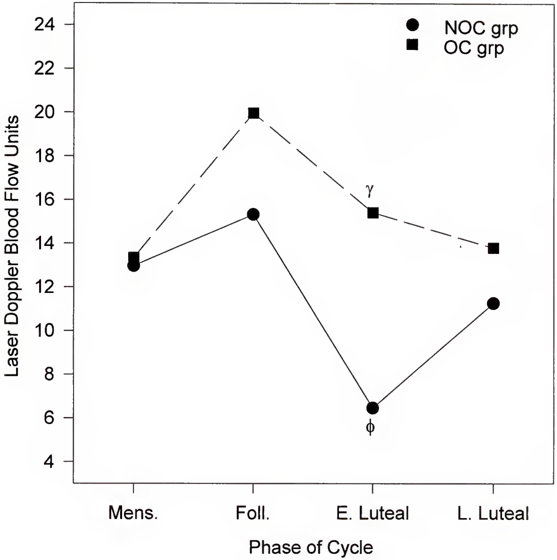

Figure 3-9. Skin blood flow across phases. Laser Doppler blood flow across phases in the NOC and OC groups. Values were obtained by averaging temperatures from Time 30 - Time 90 during exercise (means). ANOVA indicated a significant difference across phase in the NOC group ($p < .05$). ϕ - significantly different from follicular phase($p < .05$). γ - significant difference between groups ($p < .05$).

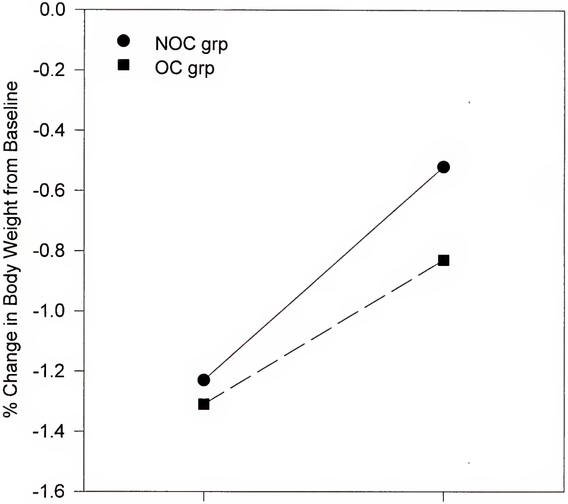

Figure 3-10. Percent change in body weight during experimental trial. The mean changes in body weight following exercise and rehydration for both the NOC and OC groups are shown. Values were calculated by dividing post-ride body weight by pre-ride body weight, and by dividing post-rehydration body weight by pre-ride body weight. These values were then multiplied by 100 to obtain the percent change in weight. ANOVA did not indicate any significant differences between experimental trial sections.

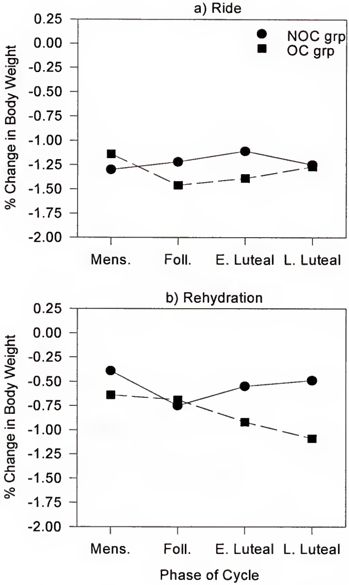

Figure 3-11. Percent change in body weight across phases. Mean changes in body weight for both the NOC and OC groups are shown. Values were calculated by dividing post-ride body weight by pre-ride body weight, and by dividing post-rehydration body weight by pre-ride body weight. These values were then multiplied by 100 to obtain the percent change in weight. a) Change in body weight following exercise across the phases. b) Change in body weight following rehydration across the phases. ANOVA did not indicate any significant differences across phases or between groups in either experimental section.

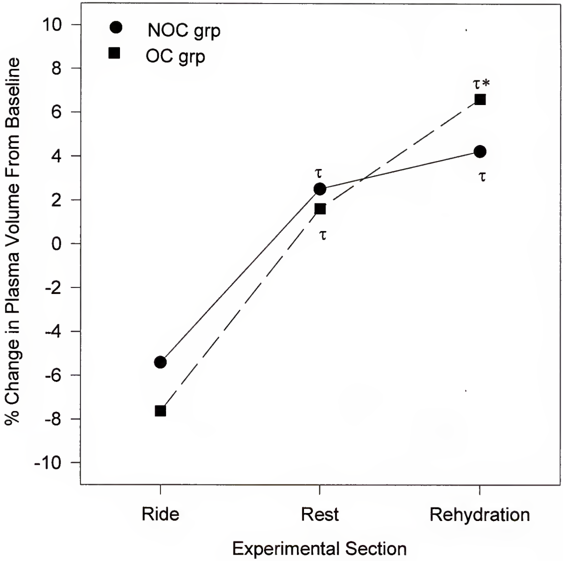

Figure 3-12. Percent change in plasma volume during experimental trial. The mean plasma volume changes following exercise, rest, and rehydration for both the NOC and OC groups are shown. Values were calculated based on hematocrit and hemoglobin changes from baseline using the formula devised by Dill & Costill (1974). ANOVA indicated a significant difference between experimental trial sections in both groups (NOC & OC $p< .001$). τ - significantly different from Ride (NOC $p< .001$; OC $p< .01$). * - significantly different from Rest (OC $p< .05$).

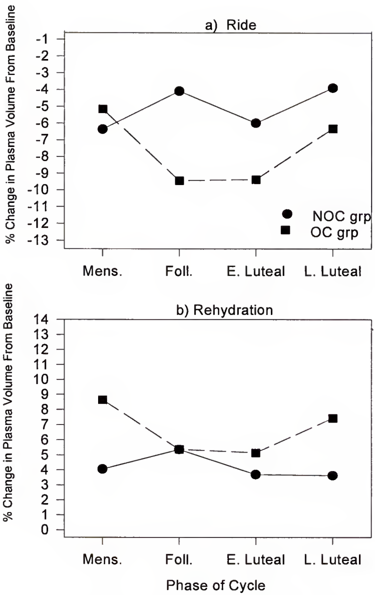

Figure 3-13. Percent change in plasma volume across phases. Mean changes in plasma volume for both the NOC and OC groups are shown. Values were calculated based on hematocrit and hemoglobin changes from baseline using the formula devised by Dill & Costill (1974). a) Change in plasma volume during exercise across the phases (Time 30 - 90). b) Change in plasma volume during rehydration across the phases (Time 150 - 240). ANOVA did not indicate any significant differences across phases or between groups in either experimental section.

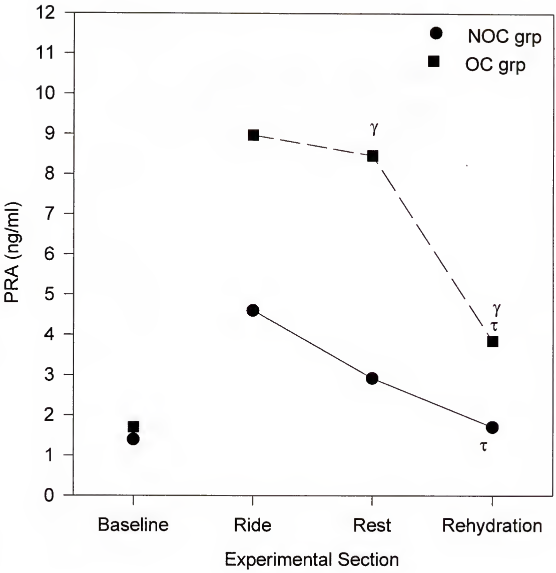

Figure 3-14. Plasma renin activity during experimental trial. Mean PRA (ng/ml) levels following exercise, rest, and rehydration for the NOC and OC groups are shown. ANOVA indicated a significant difference between experimental trial sections in both groups (NOC & OC $p< .05$). τ - significantly different from Ride (NOC & OC $p< .05$). γ - significant difference between groups ($p< .05$).

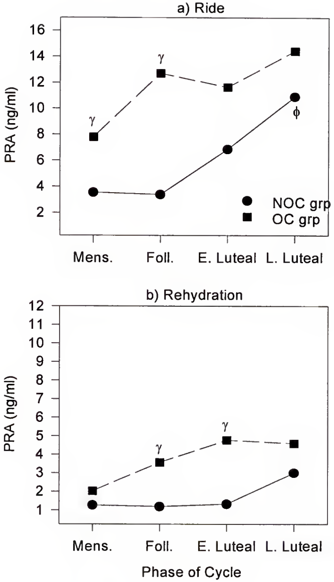

Figure 3-15. Plasma renin activity across phases. The mean PRA (ng/ml) levels across phases for both the NOC and OC groups are shown. a) Changes in PRA during exercise across the phases (mean Time 30 - 90). ϕ - significantly different from M and F phases ($p < .05$). γ - significant difference between groups ($p < .05$). b) Changes in PRA during rehydration across the phases (mean Time 150 - 240). γ - significant difference between groups ($p < .01$).

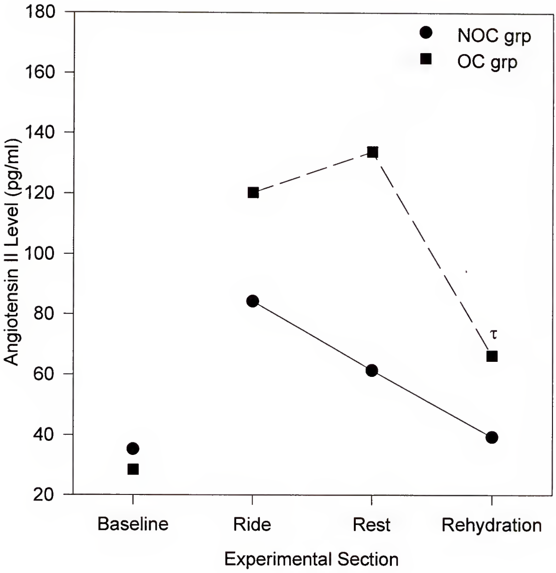

Figure 3-16. Angiotensin II levels during experimental trial. The mean ANGII (pg/ml) levels following exercise, rest, and rehydration for both the NOC and OC groups are shown. ANOVA indicated a significant difference between experimental trial sections in the NOC group ($p< .05$). τ - significantly different from Ride ($p< .05$).

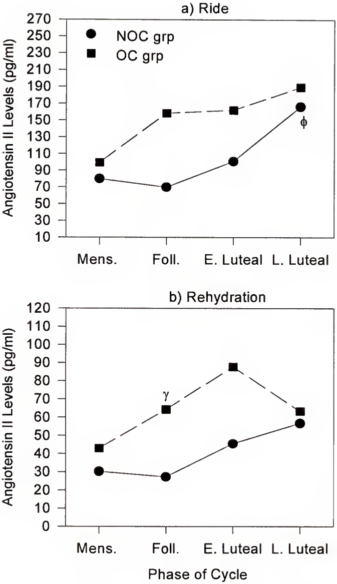

Figure 3-17. Angiotensin II levels across phases. The mean ANGII (pg/ml) levels across phases for both the NOC and OC groups are shown. a) Changes in ANGII during exercise across the phases (mean Time 30 - 90). ANOVA indicated a significant difference across phases in the NOC group ($p < .05$). ϕ - significantly different from M and F phases ($p < .05$). b) Changes in ANGII during rehydration across the phases (mean Time 150 - 240). ANOVA indicated a significant difference across phases in the NOC group ($p < .05$). γ - significant difference between groups ($p < .05$).

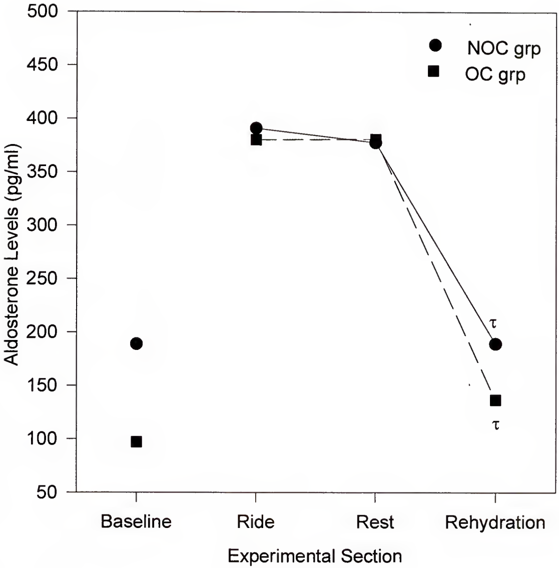

Figure 3-18. Aldosterone levels during experimental trial. The mean ALDO (pg/ml) levels following exercise, rest, and rehydration for both the NOC and OC groups are shown. ANOVA indicated a significant difference between experimental trial sections for both groups (NOC p< .001; OC p< .05). τ - significantly different from Ride and Rest (NOC p< .001; OC p< .05).

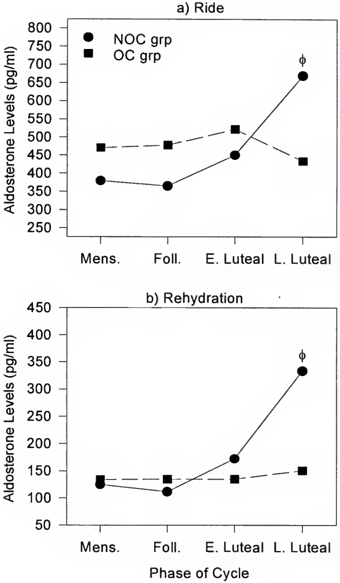

Figure 3-19. Aldosterone level across phases. The mean ALDO (pg/ml) levels across phases for both the NOC and OC groups are shown. a) Changes in ALDO during exercise across the phases (mean Time 30 - 90). ANOVA indicated a significant difference across phases in the NOC group ($p < .05$). ϕ - significantly different from M and F phases ($p < .05$). b) Changes in ALDO during rehydration across the phases (mean Time 150 - 240). ANOVA indicated a significant difference across phases in the NOC group ($p < .05$). ϕ - significantly different from M and F phases ($p < .05$).

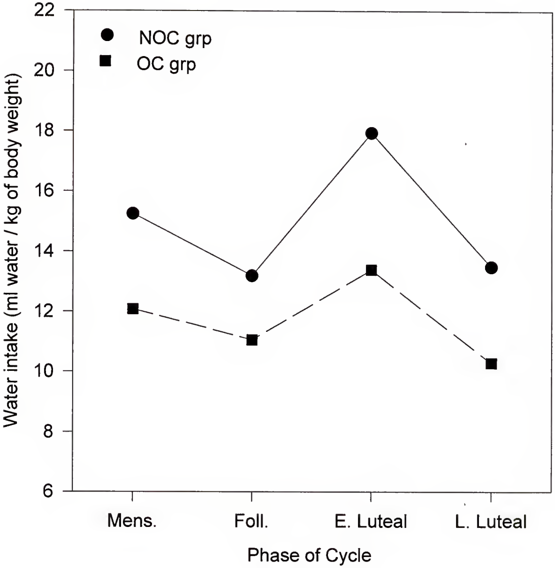

Figure 3-20. Water intake across phases of cycle. The mean water intake (ml/kg) across phases for both the NOC and OC groups are shown. ANOVA did not indicate any significant differences, either within group across phase or between groups.

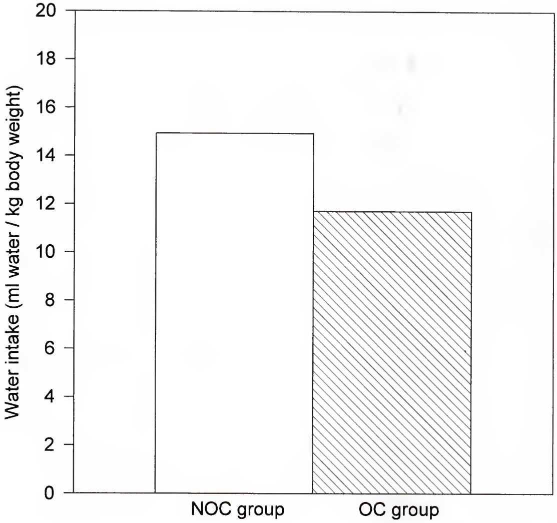

Figure 3-21. Water intake between groups. The mean water intake (ml/kg) following rehydration for both the NOC and OC groups are shown. There was no significant difference in intake between the groups.

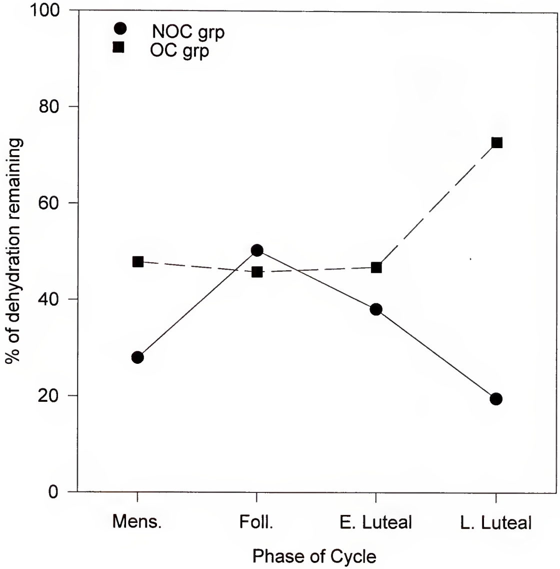

Figure 3-22. Involuntary dehydration across phases of cycle. The mean percent of involuntary dehydration across phases for both the NOC and OC groups are shown. Values were calculated by dividing the post-rehydration body weight by the post-ride body weight. These values were then multiplied by 100 to obtain the percent of involuntary dehydration remaining. ANOVA indicated no significant differences, either within group across phase or between groups.

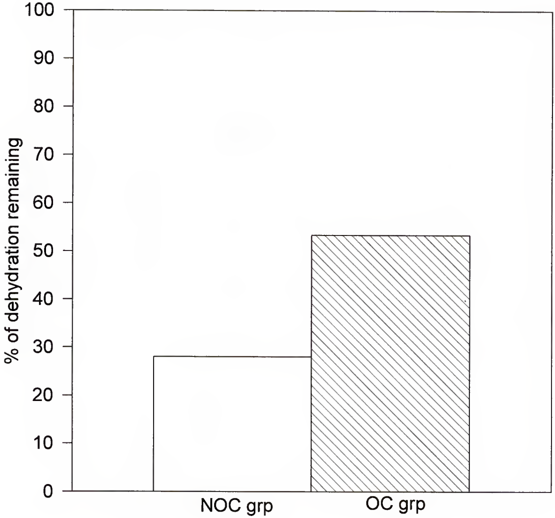

Figure 3-23. Involuntary dehydration between groups. The mean percent of involuntary dehydration remaining after rehydration for both the NOC and OC groups are shown. Values were calculated by dividing the post-rehydration body weight by the post-ride body weight. These values were then multiplied by 100 to obtain the per cent of involuntary dehydration remaining. No significant differences were indicated between the groups.

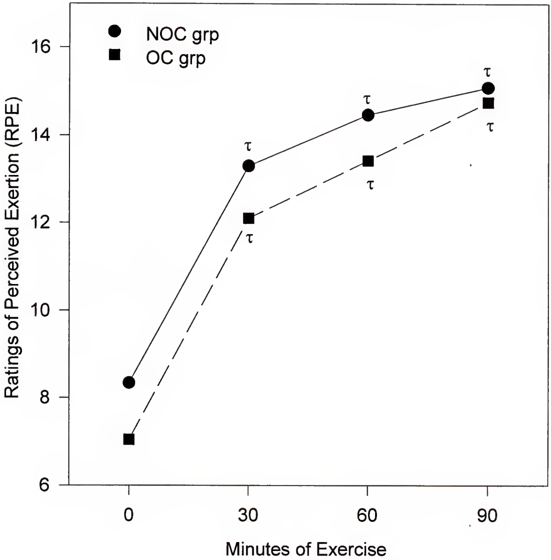

Figure 3-24. Ratings of perceived exertion during exercise. The mean RPE during exercise for both the NOC and OC groups are shown. ANOVA indicated a significant difference across time for both groups (NOC p< .001; OC p< .01). τ - significantly different from Time 0 (NOC p< .01; OC p< .05).

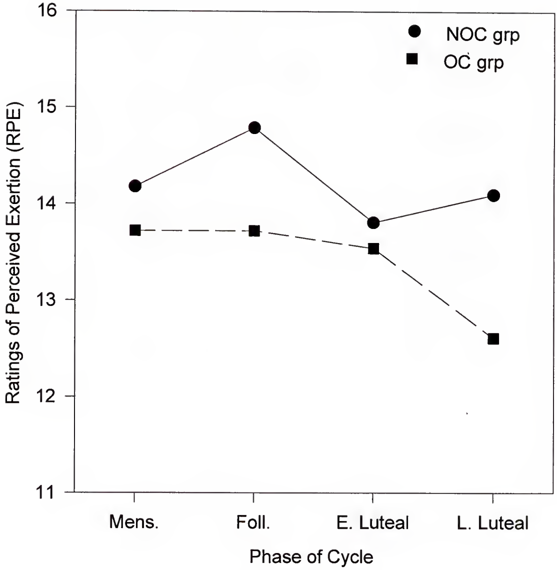

Figure 3-25. Ratings of perceived exertion across phases. The mean RPE across phases for both the NOC and OC groups are shown. Values were obtained by averaging the RPE from Time 30-90. ANOVA did not indicate any significant differences, either within group across phase or between groups.

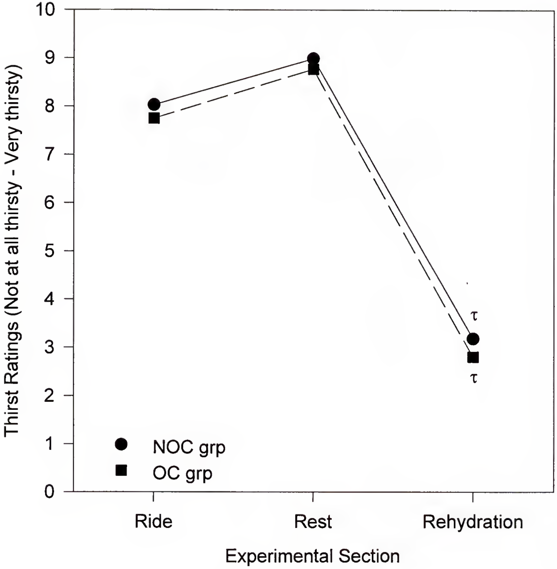

Figure 3-26. Thirst ratings during experimental trial. The mean ratings during the experimental trial for both the NOC and OC groups are shown. ANOVA indicated a significant difference between trial section for both groups (NOC & OC $p< .001$). τ - significantly different from Ride and Rest sections (NOC $p< .0001$; OC $p< .001$).

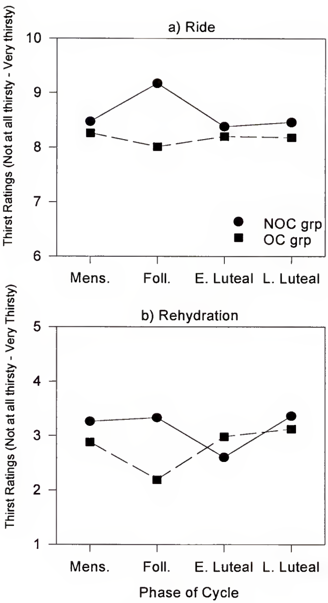

Figure 3-27. Thirst ratings across phases. The mean ratings across phases for both the NOC and OC groups are shown. a) Changes in thirst ratings during exercise across the phases (mean Time 30 - 90). b) Changes in thirst ratings during rehydration across the phases (mean Time 150 - 240). ANOVA did not indicate any significant differences, either within group across phase or between groups.

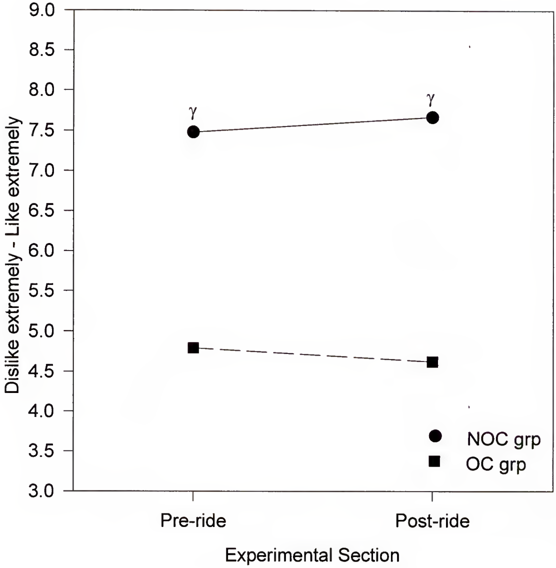

Figure 3-28. 0.01 M sodium palatability ratings during experimental trial. The mean ratings for both the NOC and OC groups are shown. ANOVA indicated a significant difference between groups ($p < .05$). γ - significant difference between groups ($p < .05$).

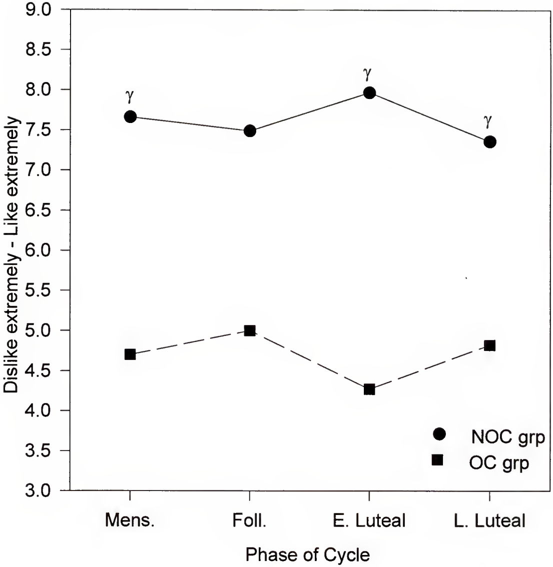

Figure 3-29. 0.01 M solution palatability ratings across phases. The mean ratings for both the NOC and OC groups are shown. ANOVA indicated a significant difference between groups ($p < .05$). γ - significant difference between groups ($p < .05$).

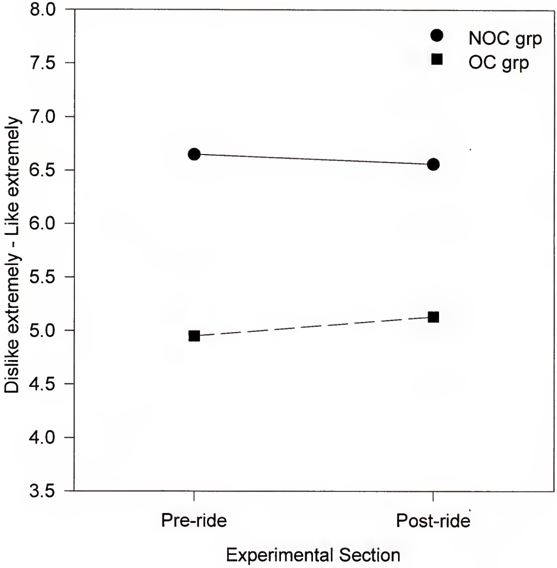

Figure 3-30. 0.03 M solution palatability ratings during experimental trial. The mean ratings for both the NOC and OC groups are shown. ANOVA did not indicate any significant differences, either within group across sections or between groups.

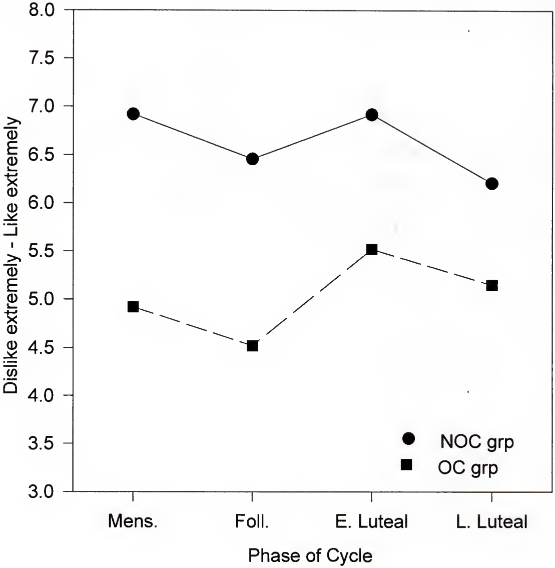

Figure 3-31. 0.03 M solution palatability ratings across phases. The mean ratings for the NOC and OC groups are shown. ANOVA did not indicate any significant differences, either within group across phases or between groups.

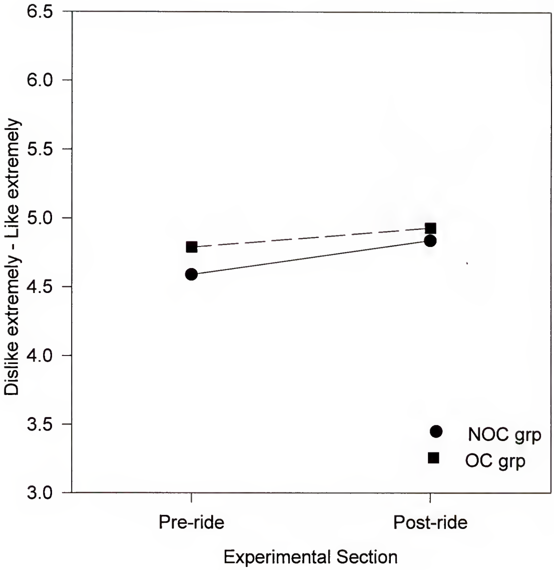

Figure 3-32. 0.06 M solution palatability ratings during the experimental trial. The mean ratings for both the NOC and OC groups are shown. ANOVA did not indicate any significant differences, either within group across sections or between groups.

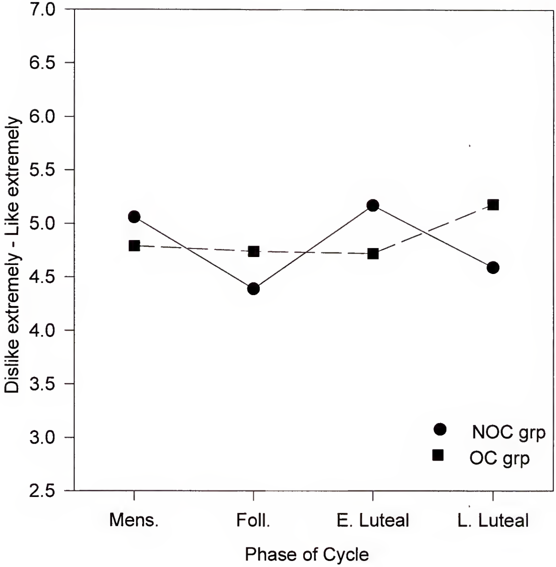

Figure 3-33. 0.06 M solution palatability ratings across phases. The mean ratings for both the NOC and OC groups are shown. ANOVA did not indicate any significant differences, either within group across phases or between groups.

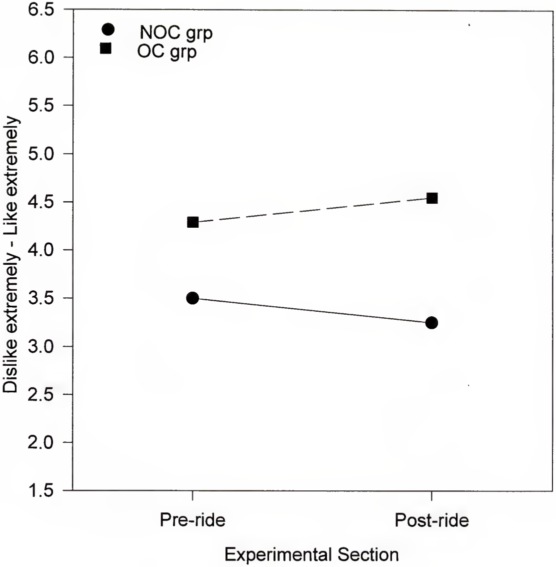

Figure 3-34. 0.1 M solution palatability ratings during experimental trial. The mean ratings for both the NOC and OC groups are shown. ANOVA did not indicate any significant differences, either within group across sections or between groups.

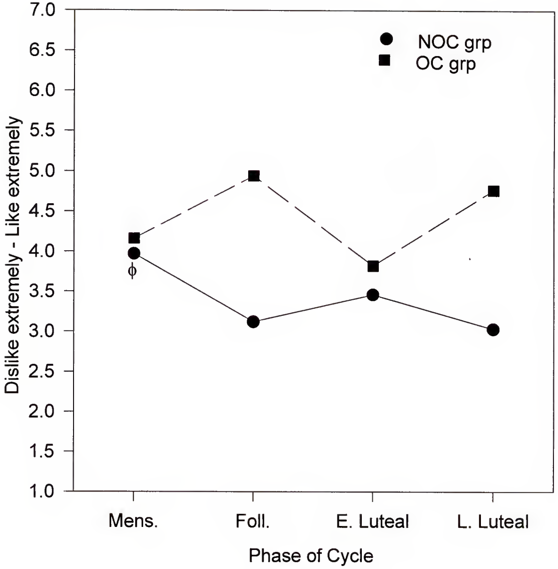

Figure 3-35. 0.1 M solution palatability ratings across phases. The mean ratings for both the NOC and OC groups are shown. ANOVA indicated a significant difference across phases in the NOC group ($p < .05$). ϕ - significantly different from F and LL ($p < .05$).

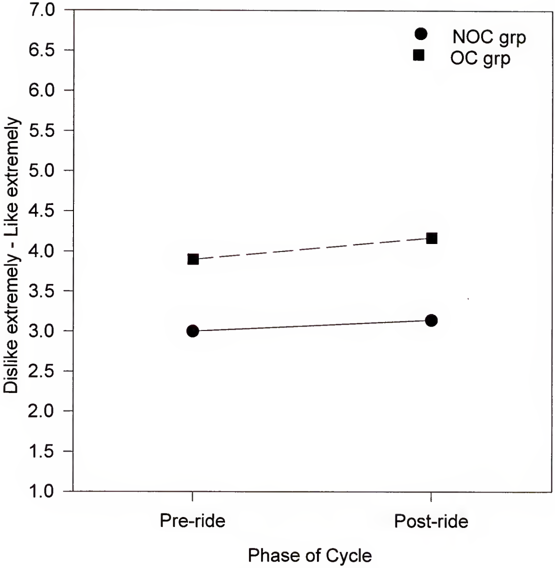

Figure 3-36. 0.15 M solution palatability ratings during experimental trial. The mean ratings for both the NOC and OC groups are shown. ANOVA did not indicate any significant differences, either within group across sections or between groups.

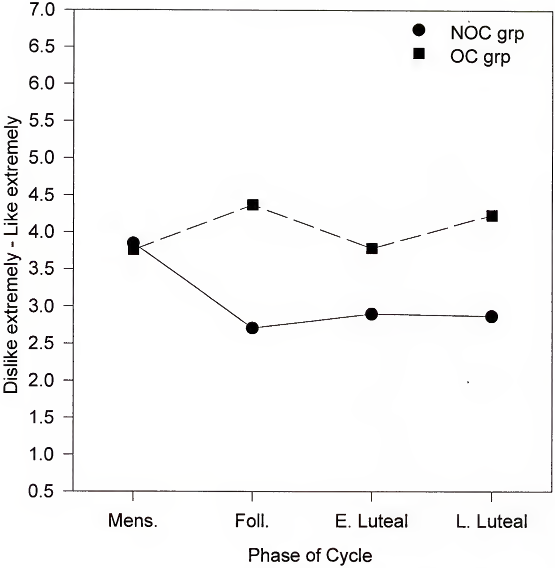

Figure 3-37. 0.15 M solution palatability ratings across phases. The mean ratings for both the NOC and OC groups are shown. ANOVA did not indicate any significant differences, either within group across phases or between groups.

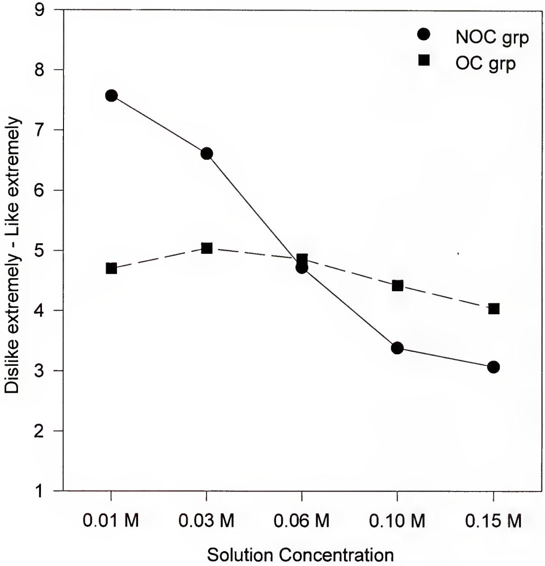

Figure 3-38. Palatability ratings for the five sodium concentrations. The mean ratings of each of the sodium solution concentrations for both the NOC and OC groups are shown. ANOVA indicated a significant difference across concentrations for the NOC group ($p < .05$) but not for the OC group.

DISCUSSION

Ovarian hormones have been shown to exert effects on thermoregulatory responses and fluid balance in females. Previous studies on exercise performance across the menstrual cycle have produced conflicting results. Some research indicates there is no significant difference in exercise responses (De Souza et al., 1990; Dombovy et al., 1987), while others have found elevated core body temperatures during exercise (Stephenson & Kolka, 1985). There is a shift in the luteal phase to initiate thermoregulatory reflexes at a higher temperature, but whether this will lead to an impairment in prolonged exercise performance is not clear. Ovarian hormones, especially estrogen, have been shown to influence water and sodium intake in animals. Although fluid intake in women has not been studied, the change in ovarian hormones across the cycle may interact with other hormones to produce a more favorable profile for rehydration in the luteal phase. In addition, the effect of the synthetic hormones in oral contraceptives on exercise performance and fluid balance has not been adequately researched. Because the pill regime consists of three weeks of exogenously administered hormones followed by one week of no hormones, changes in variables measured across the cycle should be minimized. However, there may be a slight change across the cycle comparing the menstrual phase to the other three phases. It has been noted that oral contraceptives result in a delay in the onset of thermoregulatory reflexes, similar to that seen in the luteal phase of non-oral contraceptive women. Whether this will result in higher core temperatures and impairment during endurance exercise is not known. Oral contraceptive administration in rats also is associated with changes in fluid balance and intake, yet their effects on women have not been closely examined. Women taking oral contraceptives may differ in their ingestive responses, and therefore involuntary dehydration, from normally cycling women.

This study was designed to provide information about the sensation of thirst, water intake, sodium palatability, and ability to rehydrate following endurance exercise across the cycle. The responses of normally menstruating women were compared to those using oral contraceptives. The experiments also attempted to clarify the thermoregulatory and hormonal responses across the menstrual cycle and compare them with women using oral contraceptives. Due to the difficulty in obtaining fit subjects, and the expense, the *n* for this study was small. This resulted in a lack of statistical power and difficulty in finding significant differences. Therefore, the following discussion will focus on trends observed, in addition to the significant differences detected.

NOC Group

The expected thermoregulatory and vascular reflexes occurred in response to exercise. The core, skin temperatures, and skin blood flow increased, while plasma volume decreased. The ratings of perceived exertion increased throughout the ride, and the sensation of thirst was also high. After the 30-minute rest section the plasma volume was slightly expanded from the baseline. This expansion may be due to the increased return of plasma proteins from lymphatic fluid to the vascular space that occurs during exercise (Senay & Pivarnik, 1985). Plasma proteins are forced out during exercise, and when returned, help to draw water from the interstitial space and increase plasma volume. Plasma renin activity, ANGII, and ALDO were also elevated, as expected, by exercise. The activation of the RAS and ALDO also helps to retain water and sodium, and may play a role in the post-ride hypervolemia (Fellmann, 1992). Some of the phasic changes seen in previous studies were replicated in this study. The most widely accepted changes are the increases in PRA, ANGII, and ALDO levels during the luteal half of the cycle. The exercise response of these hormones also was magnified during this time. Although no significant temperature changes were observed, a decrease in skin blood flow during the luteal phase was found. This result has been previously reported and is thought to be due to progesterone, either by increasing vasoconstriction or shifting thermoregulatory reflexes (Bartelink et al., 1990). Another possibility may be changes in plasma volume across the menstrual cycle. Turner and Fortney (1984) and Trigoso et al. (1996) have suggested that plasma volume is decreased during the luteal phase, probably due to the progesterone-induced natriuresis. The plasma volume changes in the current study showed variations across the cycle, but none were significant. However, plasma volume decreased slightly less in the late luteal phase than in the menstrual or early luteal phases. This, along with the reduced skin blood flow and elevated RAS and ALDO levels, are consistent with the presence of a contracted plasma volume. The plasma volume in the follicular phase also demonstrates a smaller change from baseline. This result may be related to the pre-ovulatory fluid retention associated with the increase in estrogen and ADH concentrations (DeVries et al., 1972; Forsling et al., 1981).

Despite fluctuations across cycle phases in some of the physiological measures during exercise, there were few significant differences and no change in the RPE.

Although many of these findings agree with previous research on exercise across the menstrual cycle, some - like plasma volume changes, bear further investigation. The number of subjects used in this study was small, which led to a lack of statistical power. In addition, there was a lack of control over the hydration status of the individuals. Although the subjects were asked to refrain from food and fluids for 12 hours before the study, there was no attempt to maintain a consistent level of hydration or solute intake prior to that. These problems may have prevented any changes in plasma volume from being statistically significant. Future studies should control for this factor and also use a technique such as Evan's Blue, which permits the measurement of absolute plasma volume as well as changes in plasma volume during exercise.

With regard to ingestive behavior, none of the expected cyclic changes occurred. Even with the hormonal changes and slight difference in plasma volume suggesting the presence of hypovolemia during the luteal phase, no significant difference in thirst, water intake or rehydration was observed. The thirst ratings for the NOC group across phase (Figure 3-27) do show small variations in the follicular and early luteal phases, during the ride and rehydration, respectively. It would have been beneficial to attempt to correlate water intake with hormone levels by including estrogen measurements. An increase during the follicular phase when estrogen dominates is unexpected and contrary to previous research. The most likely explanation is the small *n* of this study. Having only four subjects allows the mean to be greatly influenced by an outlier or single subject. A second possibility is that a high thirst rating may not necessarily be predictive of the amount of water that the person will ingest. In fact, a *post hoc* analysis did not find a significant correlation between the subjects' reported thirst ratings immediately prior to the start of the rehydration section and the total amount of water they ingested. This is probably because water ingestion quickly removes the osmotic drive for thirst. Water intake was highest during the early luteal phase. Although an enhanced water intake at this time would fit with the data suggesting a slight hypovolemia, there was a large amount of intersubject variability. It is also possible that the strain of participating in exercise influenced the subjects' perception of thirst. Future studies should examine if there is a correlation between thirst and exercise exertion or cardiac output. Water intake, degree of involuntary dehydration did not vary significantly across the phases.

The palatability of the 0.1 M sodium concentration was significantly greater in the menstrual phase. Although OXY levels vary widely across the phase, some researchers have reported an elevation in OXY levels when estrogen is high (Kumaresan et al., 1983; Stock et al., 1991). With estrogen levels at a nadir during the menstrual phase, an elevation in sodium palatability may be related to low OXY levels. Measuring plasma OXY levels at the same time sodium palatability is assessed will be important in determining if a correlation exists. However, why the palatability of a high concentration of sodium would increase while lower sodium concentration palatability does not change is unknown. It may be that the elevation of this concentration during the menstrual phase is artificially elevated by one subject's responses.

Increasing the number of subjects in future experiments would help reduce this variability, yet other factors may be influencing intake. Szlyk et al. (1989b) has classified subjects as either reluctant or avid drinkers. The reluctant drinkers in their study drank 31% less water than the avid drinkers. They reported that the two groups of subjects had similar backgrounds, but did not define what that meant. Therefore, it is possible that different experiences may influence subsequent water intake, in particular, type of exercise. People who primarily engage in long distance sports, such as marathon running or cycling, may have learned to drink more and rehydrate better compared to shorter distance exercise. The current study did not attempt to classify reluctant versus avid drinker or control for type of exercise the subject normally engaged in. In the future, it will be interesting to survey people on their knowledge of rehydration and attempt to correlate that with exercise-mode background.

Recent research indicates that the sodium palatability tests were conducted too soon after exercise to show an increase in palatability. A study by Takamata et al. (1994) showed that the palatability ratings for low concentrations of NaCl did not increase until one hour after exercise, whereas in the current study only 30 minutes elapsed. This would also prevent more phasic changes in sodium palatability from being identified. In future studies, sodium palatability ratings should be assessed over a much longer period of time, even up to 24 hours.

OC group

The exercise response of the OC group was similar to that of the NOC group. Core temperatures, covered skin temperatures, and SBF increased and plasma volume decreased during exercise. Across the 90 minutes of exercise, the ratings of perceived exertion increased and the thirst ratings were high. Similar to the NOC group, the plasma volume of the OC group returned to a slightly elevated level by 30 minutes post-exercise. Again, this result is probably due to an increase in plasma protein return and the high levels of hormones important in water and sodium retention. As expected, the levels of

PRA, ANGII, and ALDO were increased in response to exercise, and remained high during the rest section.

The OC group had no significant changes across the phases in the exercise responses. However, there were some trends observed. Core and skin temperatures were slightly higher during the luteal phase, probably as a result of the increased progestin levels. In the follicular phase, skin blood flow was slightly increased. This may be the result of an outlier because of the small *n*, but it also could be an effect of oral contraceptive intake. During the follicular phase, estrogen is dominant and may exert a vasodilating effect (Bartelink et al., 1990). It is conceivable that similar things are happening in OC group with the women taking the phasic combination pills. When the estrogen component of the pill is dominant, the thermoregulatory threshold for heat loss response may be reset downward. This threshold shift could produce vasodilation and a higher skin blood flow.

Oral contraceptives have been found to increase capillary permeability and plasma volume in women (Tollan et al., 1992). Increased permeability may allow more fluid to be shifted out of the vasculature during exercise. The current study found the volume in the menstrual phase decreased the least, suggesting that the synthetic hormones are affecting the vascular fluid dynamics. The decrease in plasma volume during exercise is greater in the follicular and early luteal phases. The high skin blood flow could explain the greater fluid loss in the follicular phase, but is slightly lower in the luteal phase. Triphasic combination pills have the highest progestin concentration during the late luteal phase. Many of the oral contraceptive preparations use a synthetic progesterone which does not have a natriuretic effect, and therefore, promotes fluid retention. Therefore, the women using triphasic preparations may have a greater absolute plasma volume later in the cycle. This would allow them to lose a smaller percentage of their plasma volume, but the same absolute amount. It is interesting to note that the RPE of the OC group are lowest during the late luteal phase. Ratings of perceived exertion have been correlated with heart rate, and a larger plasma volume helps maintain cardiac output at a lower heart rate. Future studies may be able to produce clearer effects if the type of progestin and phasic make-up of the oral contraceptives were consistent between subjects, and measure heart rate and absolute plasma volume.

Differences in the levels of the measured hormones from the menstrual (no hormone) phase compared to the rest of the phases also indicates an effect of oral contraceptives. This result was expected, as oral contraceptive use has been found to slightly increase PRA and ANGII levels. Aldosterone levels did not change significantly across the phases, which is consistent with the types of progestins used by these subjects. These synthetic progesterones do not have a natriuretic effect, and therefore would not stimulate ALDO secretion.

The OC group did not have any statistically significantly changes across the phases, although some trends were observed. Previous studies also have reported the slight increase in RAS activation with oral contraception. In addition, some researchers have suggested that plasma volume and capillary permeability also are increased. In the present study, the direction of change in these variables tended to support those findings. Including more subjects, controlling for the type of oral contraceptive used, and measuring absolute plasma volume would have helped to produce clearer results and possibly a stronger effect.

There were no significant differences across phases for any of the ingestive measures of the OC group. A decrease in thirst ratings occurred in the follicular phase, and could potentially be related to estrogen administration. There was a slight increase in water intake during the early luteal phase, similar to the one seen in the NOC group. However, the large degree of intersubject variability and the small number of subjects makes drawing any conclusions difficult. Following the suggestions made in the section discussing the NOC group data would benefit future research conducted on the effect of oral contraceptive administration on ingestive behaviors.

NOC vs. OC

In the comparison of measures between the groups, many differences were observed with several reaching statistical significance. The core temperatures during the ride were similar between the two groups. The difference in core temperature during the early stage of the ride was only slightly more than a tenth of a degree, and may be explained, in part, by variations in the amount of activity between subjects immediately prior to the experimental trial. Although it did not reach significance, the NOC group had consistently higher skin temperatures than the OC group throughout exercise. Other studies have reported higher core and skin temperatures in women taking oral contraceptives than when they were not on the pill (Grucza et al., 1993; Rogers & Baker, 1997), so this outcome was unexpected. There are several possible interpretations of this result. The cutaneous vasodilation induced by exogenous estrogen may be responsible for the lower core temperature in the OC group (Brooks et al., 1997). Also, the previous studies followed the same subjects while on and off oral contraceptives. The current study compared two separate groups of subjects, so intersubject variability may have obscured any slight trends in oral contraceptive effects on body temperatures.

Another explanation may be related to differences in skin blood flow and plasma volume between the groups. Skin blood flow, particularly in the last 30 minutes of exercise is much lower in the NOC group than the OC group. By maintaining high skin perfusion during exercise, the OC group is better able to use evaporative heat loss. The similar core temperatures between the groups and the higher skin temperatures of the NOC group suggests that in the cool ambient temperature of these experiments, they are able to compensate for the lower skin blood flow. As has been previously seen (Hommen et al., 1990), the core temperature in the NOC group may have been high enough to increase the amount of heat carried by each unit of blood. In addition, having higher skin temperatures increases the skin to ambient temperature gradient and facilitates heat loss through convection and radiation.

The ovarian hormones and plasma volume can affect cutaneous vasodilation. Hormone influence, either direct or indirect, is indicated by the similar level of skin blood flow during exercise for both groups in the menstrual cycle. This is the week of the cycle when the OC group takes the inert pills. Exogenous estrogen has been shown to increase cutaneous vasodilation, but the addition of progestins blocks this effect (Brooks et al., 1997). It may be that phasic pills with a low progestin to estrogen ratio early in the regime do have a net effect toward vasodilation in the OC group.

However, changes in plasma volume may be more important in explaining group differences, particularly in the later half of the cycle. The OC group lost more plasma volume during the ride in all phases but the menstrual. The greater plasma loss could be related to increased capillary permeability in the OC group, but can also be associated with a greater absolute plasma volume. Some studies have indicated that women taking combination oral contraceptives have an increased cardiac output probably related to an expanded plasma volume. A greater plasma volume in the OC group compared to the NOC group would allow them to maintain the higher skin blood flow during exercise found in this study. Having a larger vascular volume would also permit the OC group to shift more fluid out during exercise without a deleterious effect on cardiovascular functioning. The lower temperatures, higher skin blood flow, and presumed increase in plasma volume that is suggested by these results may facilitate exercise performance in the OC group. Although the differences were not statistically significant, the RPE of the OC group was slightly lower than that of the NOC group.

As suggested by the increase in RAS precursors by oral contraceptives, the hormonal response of this group was greater than the NOC group. The largest differences were observed during the follicular and early luteal phases, after which the NOC group levels of PRA, ANGII, and ALDO rose. The increased RAS activation is probably due to the greater availability of plasma renin substrate and angiotensinogen when exercise begins, so that more angiotensin I can be produced and converted into ANGII. Also, the larger plasma volume loss of the OC group may more strongly stimulate RAS activation and ALDO release than the NOC group.

Despite the greater plasma volume loss and higher hormone levels in the OC group, there were no apparent differences in thirst ratings between the groups (Figures 3-26 and 3-27). Even so, the OC group drank less water and had a greater degree of involuntary dehydration remaining than the NOC group. The small *n* and large variability in responses prevented any significance from being detected. Including the plasma osmolality and protein measurements of both groups would have provided valuable information for evaluating thirst and water intake in this study. Although, the OC group drank slightly less than the NOC group, their plasma volume was slightly greater following rehydration. If the OC group returns more plasma proteins to the vascular space and draws more fluid back in or has a lower plasma osmolality, the stimulus for water intake may be reduced compared to the NOC group. However, the observed differences in plasma volume between the groups may be the result of very high responses in one OC subject.

The sodium palatability ratings showed that the NOC group found the lowest concentrations (0.01 and 0.03 M) more acceptable than the OC group. The decrease in palatability may be related to OXY levels. As mentioned in the literature review, OXY levels are elevated in oral contraceptive users. Besides being important in facilitating labor and breast-feeding, this hormone is also associated with inhibiting sodium appetite. An attempt was made to measure the OXY levels of subjects in this study, but the samples became overheated and degenerated. It will be interesting to attempt to correlate sodium palatability and OXY levels in a future study. Carrying the sodium palatability test out over a longer period of time may reveal further changes in palatability between OC and NOC women. The NOC group also showed a significant decrease in sodium palatability as the sodium solution concentration increased, while the OC group's palatability ratings remained relatively stable. As mentioned in the Results section, one of the OC subjects had higher palatability ratings than the other two subjects. This caused the average to remain high instead of decreasing as the sodium concentration increased. Including a greater number of subjects in future palatability studies will help to decrease the effect of extreme values from one subject.

Summary

No changes in exercise performance across menstrual cycle phase were found in either NOC or OC subjects. The only significant change in any of the measures followed during exercise was for SBF in the NOC group. These results are similar to other studies that have found that exercise performance is not significantly affected by cycle phase. There is, however, an elevated exercise response in PRA, ANGII, and ALDO during the luteal phase of the NOC group. In both groups, the levels of these hormones were not significantly different from baseline levels after RHYD. The sodium palatability of one concentration was elevated during the menstrual phase. This result may be related to lower OXY levels at this time and could promote rehydration with sodium solutions at this time. Involuntary dehydration in men after 3 hours has been reported to be approximately 50% (Greenleaf & Sargent, 1965), whereas the present study produced a combined average of 40% in both groups of women after 2 hours of rehydration. Further studies using a greater number of subjects will have to be conducted to determine whether or not a gender difference exists with regards to involuntary dehydration.

This study also compared the exercise and ingestive responses of NOC and OC women. The trends in the physiological variables measured during exercise suggest that oral contraceptive usage may facilitate exercise performance, probably by reducing cardiovascular strain. Enhancing cardiac output lowers the RPE during exercise, but whether or not it will affect the duration or quality of exercise performance is unknown.

There were interesting differences indicated by the ingestive measures that suggest the OC group had slightly impaired rehydration. If oral contraceptive use reduces water intake or the acceptability of rehydration solutions containing sodium, it could delay rehydration or result in a cumulative fluid deficit with repeated exercise bouts. This could, in turn, impair performance.

Several modifications should be made in future studies examining ingestive behavior in women. The most obvious is including a larger number of fit subjects. Controlling the hydration status of the subjects and the type of oral contraceptive that they are using will help to reduce intersubject variability. Subjects may also need to be grouped by category of drinker (reluctant vs. avid). Measuring absolute plasma volume, proteins, and osmolality, along with estrogen and oxytocin levels will permit correlating the physiological status of the subject with their ingestive measures.

APPENDIX A
HEALTH AND EXERCISE SCREENING QUESTIONNAIRES

DEMOGRAPHIC INFORMATION

NAME_____________________________________ **DATE** ____/____/____
Last First MI Month Day Year

RESIDENCE ____
Street

____
City State Zip

SOCIAL SECURITY #____-____-____ **PHONE #**________________(H)

________________(W)

AGE__________ **DATE OF BIRTH**____/____/____
Month Day Year

HEIGHT ________inches **WEIGHT** _______lb.

Race
_____White _____Black _____Asian _____Latino _____Other:_______________

MEDICAL HISTORY

Answer the following questions, indicating the month and year of the event of diagnosis where appropriate.

	Yes	No
1. Has a doctor ever told you that you have heart disease?	_____	_____
2. Do you have hypertension (high blood pressure)?	_____	_____
3. Do you have diabetes mellitus?	_____	_____
4. Has your doctor ever told you that you have a heart valve problem?	_____	_____

5. If yes to any of the above, explain:______________________________________

____

____

6. OTHER MEDICAL PROBLEMS: Indicate if you have had any of the following medical problems:

Past	Now	
______	______	Alcoholism
______	______	Allergies
______	______	Anemia
______	______	Arthritis
______	______	Asthma
______	______	Back injury or problem
______	______	Blood clots
______	______	Bronchitis
______	______	Cirrhosis
______	______	Claudication
______	______	Elbow or shoulder problems
______	______	Eye problems
______	______	Gall bladder disease
______	______	Glaucoma
______	______	Gout
______	______	Headaches
______	______	Hemorrhoids
______	______	Hernia
______	______	Hip, knee or ankle problems
______	______	Intestinal disorders
______	______	Kidney disease
______	______	Liver disease
______	______	Lung disease
______	______	Neurologic disorder
______	______	OB/GYN problems
______	______	Phlebitis
______	______	Rheumatic fever
______	______	Seizure disorder
______	______	Stomach disease
______	______	Thyroid disease
______	______	Tumors or cancer - List type: ________________________________
______	______	Ulcers
______	______	Other - specify: ______________________________________

If yes to any of the above, explain:____

____

____

7. **SURGICAL PROCEDURES**: Indicate if you have had any of the following surgeries.

Yes	No	
______	______	Adhesion repair
______	______	Appendectomy
______	______	Back surgery

Yes	No	
_____	_____	Bladder surgery
_____	_____	Bowel surgery
_____	_____	Breast surgery
_____	_____	Cataract surgery
_____	_____	Gall bladder surgery
_____	_____	Hemorrhoid surgery
_____	_____	Joint surgery
_____	_____	Kidney surgery
_____	_____	Lung surgery
_____	_____	OB/GYN surgery
_____	_____	Stomach surgery
_____	_____	Other - specify: ______________________________

If yes to any of the above, explain:______________________________

____

____

8. **MEDICATIONS**: Indicate the medicines you currently use on a regular basis.

Yes	No	
_____	_____	Allergy medicines/antihistamines
_____	_____	Antacids
_____	_____	Antibiotics
_____	_____	Anti-arrhythmics
_____	_____	Anti-inflammatory agents
_____	_____	Aspirin
_____	_____	Asthma medicines
_____	_____	Beta blockers
_____	_____	Birth control pills (# of years: _____0-1 _____1-5 _____5-10 _____10+
_____	_____	Blood pressure medicines
_____	_____	Blood thinners
_____	_____	Cortisone
_____	_____	Diabetes medicines/insulin
_____	_____	Diuretics/"water pills"
_____	_____	Gout medicines
_____	_____	Heart medicines
_____	_____	Hormones/estrogen
_____	_____	Laxatives
_____	_____	Nitroglycerin
_____	_____	Pain medicines
_____	_____	Psychiatric medicines/anti-depressants
_____	_____	Sedatives/sleeping pills
_____	_____	Seizure medicines
_____	_____	Thyroid medicines
_____	_____	Tranquilizers
_____	_____	Vitamins/iron
_____	_____	Other - specify:______________________________

FAMILY HEALTH HISTORY

1. If any members of your immediate family have or have had any of the following conditions, indicate their age at the time of the event:

	Father	Mother	Brother(s)	Sister(s)
Heart Attack	______yr	______yr	______yr	______yr
Stroke	______yr	______yr	______yr	______yr
Coronary Artery Disease	______yr	______yr	______yr	______yr
If deceased, note age at time of death	______yr	______yr	______yr	______yr

2. Indicate if any members of your immediate family have or have had the following conditions by marking the appropriate lines.

	Father	Mother	Brother(s)	Sister(s)
High Blood Pressure	______yr	______yr	______yr	______yr
High Cholesterol	______yr	______yr	______yr	______yr
Diabetes	______yr	______yr	______yr	______yr
Obesity	______yr	______yr	______yr	______yr

ACTIVITY STATUS

1. Please indicate your usual activities.

	Frequency per month					Minutes per session			
	1-4	5-8	9-12	13-16	17+	0-20	20-40	40-60	60+
___ Badminton	____	____	____	____	____	____	____	____	____
___ Baseball/softball	____	____	____	____	____	____	____	____	____
___ Boating	____	____	____	____	____	____	____	____	____
___ Bowling	____	____	____	____	____	____	____	____	____
___ Cycling (motor)	____	____	____	____	____	____	____	____	____
___ Cycling (road)	____	____	____	____	____	____	____	____	____
___ Cycling (stationary)	____	____	____	____	____	____	____	____	____
___ Dancing (aerobic)	____	____	____	____	____	____	____	____	____
___ Dancing (social)	____	____	____	____	____	____	____	____	____
___ Golf (ride)	____	____	____	____	____	____	____	____	____
___ Golf (walk)	____	____	____	____	____	____	____	____	____
___ Gymnastics	____	____	____	____	____	____	____	____	____
___ Hiking	____	____	____	____	____	____	____	____	____
___ Horseback riding	____	____	____	____	____	____	____	____	____
___ Hunting, fishing	____	____	____	____	____	____	____	____	____
___ Jogging/running	____	____	____	____	____	____	____	____	____
___ Martial arts	____	____	____	____	____	____	____	____	____
___ Racquet/handball	____	____	____	____	____	____	____	____	____
___ Rope jumping	____	____	____	____	____	____	____	____	____
___ Rowing, canoeing	____	____	____	____	____	____	____	____	____
___ Sailing	____	____	____	____	____	____	____	____	____

Gynecological History Form
University of Florida
Gainesville, FL 32611

Date: ____________
Name: ______________________________ Age: ________
Address: ______________________________
____

1. MENSTRUAL HISTORY:

How old were you when your periods began? ____________
How often do you have your period? ____________
Have you had any problems with your periods in the past? ____________________
____
Do you take any medication for menstrual cramps? ________ What? ____________

2. GYNECOLOGY HISTORY:

Date of last Pap Smear ____________ Pelvic exam ____________
Have you ever had an abnormal Pap smear? ________ When? ____________
What follow up was done? ______________________________

Have you had any of the following gynecology problems?

	✓
1. Ovarian cyst	
2. Unusual vaginal bleeding	
3. Breast problems or surgery	
4. Gynecological surgery	
5. Frequent vaginal infections	
6. Frequent bladder infections	

4. OBSTETRICAL HISTORY: Have you ever been pregnant? ____________

	Term Births	Premature Births	Miscarriage or Abortion	Live Children
Number of:				

Did you have any problems with any of the above? ________ If yes explain. ____________
____

Do you have any other additions to your history which would be helpful? ____________
____

APPENDIX B
THIRST RATINGS

Subject #__________ Date____________

Trial______________ Time____________

How thirsty do you feel now?

Very thirsty ____ Not at all thirsty

APPENDIX C
SODIUM SOLUTION RATINGS

Solution Ratings

Date__________________

Subject___________________________________Subject #__________

Trial____________________________________ Time____________________________

Solution:

______A How would you rate the taste of this solution?

Dislike extremely ____ Like extremely

______B How would you rate the taste of this solution?

Dislike extremely ____ Like extremely

______C How would you rate the taste of this solution?

Dislike extremely ____ Like extremely

______D How would you rate the taste of this solution?

Dislike extremely ____ Like extremely

______E How would you rate the taste of this solution?

Dislike extremely ____ Like extremely

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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